STUDIES ON THE EFFECT OF SOME PESTICIDES ON BLOOD PARAMETERS IN FRESH WATER FISHES OF BUNDELKHAND REGION

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Under guidance of
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Time swallows up

Everything that is visible,

Sparing nothing.

It does not spare even

Outstanding personalities.

This Thesis is **Dedicated** in words and spirits to my revered Grand Father

Late Shri Maiyadeen

Dr. Kaneez Zahra
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Date 4/11/09

Certificate

This is to certify that the thesis entitled **"Studies on the effect of some pesticides on blood parameters in fresh water fishes of Bundelkhand region"** embodies the original research work of **Saurabh Shreshth** who worked under my supervision and guidance for more than five years in the department of Zoology, Bipin Bihari (P.G.) College, Jhansi.

This thesis has not been submitted for any degree to any other university.

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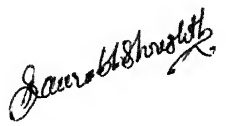


(Saurabh Shreshth)

Chapter - 1

INTRODUCTION

INTRODUCTION

The study of Toxicology has generated greater excitement and become a subject of general interest in the present century. Hippocrates was the first to introduce primitive principals of toxicology around 400 BC. More significant contributions of Toxicology were made in 16th century and Paracelsus (the father of toxicology) was the first to introduce the wide theory of Toxicology. Toxicology is derived from the word toxicum means poison, and logus means knowledge i.e. "the science of poison". It can be defined as, "A discipline of science which deals with the various aspects of poison and poisoning is known as Toxicology". Poison may be defined as a substance which even in small dose produces adverse effects in metabolism of an organism and consequently may cause death.

Toxicology is the study of how specific chemicals cause injury to living cells and organism. It is a study to determine how easily the chemical enters the organism, what cells are affected by the chemicals and what cell functions are impaired. Toxicological study is becoming an increasingly important subject of modern society because of the adverse effects of the growing use of chemicals and high-tech radioactive appliances. With increasing population, the modern society demands improvement of the health and living conditions such as nutrition, clothing, dwelling and transportation. In order to fulfill these growing needs a large variety of chemicals must be manufactured and used. These chemical compounds come in contact with various segments of the population (animal and human beings) directly or indirectly causing adverse effect on them.

Toxicology is multi disciplinary science. It includes Environmental, Economic, Clinical and Forensic toxicology. The environmental degradation due to the presence of various pollutants is known as environmental toxicology. My research topic is related to the environmental toxicology. Environmental toxicology is normally based on the pollution studies. In general pollution can be defined as a matter in wrong place or any substance released into the environment which degrades it. Our environment becomes continuously polluted due to continued economic growth, mismanagement of resources, population growth and more use of toxicants (Stout B.A., 1976; Kutty et al., 1977; Suege J. and Bluzat R., 1983; Kulshrestha S.K. and Arora A., 1984; PAN Pesticide Database 1996; Kumar Suresh et al., 1999). A toxicant may be defined as an agent that causes adverse effect or response in biological system, seriously damaging its structure or function or producing death. The adverse effect or response may be defined in the term of a management that is out side the normal range for healthy organism. The toxicants are released from various sources into air, water and soil. They get into human food chain from the environment. Once the toxicants enter our biological system, they interfere with the biochemical process as may lead to fatal results.

Aquatic Toxicology: -

A large number of toxicants reach in the aquatic environment. The sources of toxicants in aquatic environment are :-

- Sewage and other wastes of domestic nature
- Agricultural discharges

- Industrial effluents
- Wastes from thermal and nuclear power plants

Aquatic toxicants may be grouped into two categories

1. Toxic trace elements and heavy metals found in natural and wastes water,
2. Pesticides

Classification of pesticides:-

A pest is described as the trouble some or destructive organism and pesticides may be defined as substances intended for killing pest. Pesticides may be classified in various ways

1. **Classification of pesticides by target organism:-**

Acaricides : Used against mites and ticks

Algaecide : Used against algae

Fungicides : Used against fungi

Molluscicide : Used to kill molluscs pest and other invertebrates

Nematicide : Used against Nematodes

Herbicides : Weed kills

Insecticides : Used against insects

Rodenticides : Used against mice, rats and other rodents

2. **Classification on the basis of chemical classes:-**

On the basis of chemical nature, the pesticides may be broadly divided into two groups

a. Organic pesticides

b. Inorganic pesticides

At present, the uses of inorganic pesticides are less than organic pesticides. Mostly the synthetic organic pesticides are being used in developed and developing countries. These are organochlorine, organophosphorus, natural and synthetic pyrethroide and dinitrophenols etc.

Contamination by pesticides

There are various ways through which the pesticides add into the environment either by direct or indirect applications (Heath AG., 1995; Heath AG., 1996; Braunbeck, T.,1994; Mason C.F. ,1996; Hrudey, S.E., W. Chen and C.G. Roussex, 1996).

(A) Direct application of pesticides

The pesticides are directly applied to water bodies for any of the following purposes;

(i) for the control of unwanted weeds.

(ii) for the control of insect pests infecting water plants.

(iii) for the control of insects and their life-stages of public-health importance, for instance mosquito larvae.

(iv) for the control of undesired fish in fish culture ponds to restock with more desirable fish.

(B) Indirect application of pesticides

The pesticides indirectly reach the aquatic reservoirs by any of the following ways:

(i) A heavy rainfall soon after the pesticide application in agricultural fields washes away fairly large share of the pesticides with the run off water, otherwise only a small share of pesticides reach into water bodies through agricultural run off. The water soluble pesticides are transported in dissolved state while insoluble pesticides get bound to particulate matter, which is carried by water.

(ii) Pesticides manufacturing factories may release large amounts of pesticides, even in the effluents, which are treated for the removal of pesticides.

(iii) Pesticides may also reach water- bodies on account of fall out from accidental spray from large scale aerial spraying of forest or agricultural field's. Usually major fall out occurs close to the site of their application polluting nearby water- bodies, but some times wind may carry the drift to considerable distances.

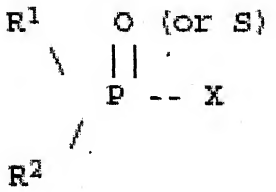
Production and Usage of pesticide in India

The word pesticide covers a broad range of compounds including insecticides, herbicides, pesticides, plant growth regulators and others. The production of pesticides started in India in 1952 with

the establishment of a plant for the production of oraganophosphorus pesticides near Calcutta, and India is now the second largest manufacturer of pesticides in Asia after China and ranks twelfth globally. There has been a steady growth in the production of pesticides in India, from 80,000 metric tones, in 1988 to 102,240 metric tones in 1998. In 2008 it became to 134,004 metric tones. The use of herbicides is correspondingly less. The most important crops with regards to insecticidal uses are cotton, fruits, vegetables, cereal and maize.

General information of toxicants:-

Several oraganophosphorus insecticides have been reviewed by WHO for consideration as agents for the control of disease vectors. A large number has been reviewed by the FAO/WHO Joint Meetings on Pesticide Residues. These compounds are designed to be toxic for certain pests and are added deliberately to the environment. Oraganophosphorus insecticides are normally esters, amides, or thiol derivatives of phosphoric, phosphonic, phosphorothioic, or phosphonothioic acids. The oraganophosphorus pesticides are derivatives of phosphoric acid having one or more, rarely two atoms of phosphorus whose one or more hydrogen atoms are placed by alkyl groups. General formula of oraganophosphorus compound is



where R1 and R2 are usually simple alkyl or aryl groups, both of which may be bonded directly to phosphorus (in phosphonates), or

linked via -O-, or -S- (in phosphates), or R1 may be bonded directly and R2, and X bonded via one of the above groups (phosphonates).

The primary biochemical effect associated with toxicity caused by oraganophosphorus pesticides is inhibition of AChE. The normal function of AChE is to terminate neurotransmission due to ACh that has been liberated at cholinergic nerve endings in response to nervous stimuli. Loss of AChE activity may lead to a range of effects resulting from excessive nervous stimulation and culminating in respiratory failure and death.

The pesticides employed to kill weeds are called herbicides or weedicides. In early 20 th century various inorganic salts were used to control weeds. But most of the herbicides are organic compounds (Kumar Hemant and Gupta A.B., 1997; Nath Ravindra, Banerajee V., 1999; Grillitsch et al., 1999; Mc. Kim J. M., and Lien G. J., 2001; Hoff et al., 2003; Mc Donald M.D. and Grosell M., 2006).

Glyphosate kills plants by inhibiting the activity of the enzyme 5- enolpyruvylshikimic acid-3-phosphate synthesis (EPSP), which is necessary for the formation of the aromatic amino acids tyrosine, tryptophan, and phenylalanine. These amino acids are important in the synthesis of proteins that link primary and secondary metabolism. Glyphosate also act as a competitive inhibitor of phosphoenolpyruvate (PEP), which is one of the precursors to aromatic amino acid synthesis. It also affects other biochemical processes, and, although these effects are considered secondary, they may be important in the total lethal action of glyphosate (Monsanto Company 1985; Sawada Y., Nagai Y., 1987; Moses M., 1989; Tai T. et al., 1990; Tominack et

al., 1991 Talbot A.R., et al., 1991; Ayoola S.O., 2008).

Choice of test organism

The introduction of pesticides in water can decrease the health of natural aquatic ecosystems. To determine the "health status" of a river or dam, it is important to consider an effective and reliable test organism. The following criteria must be taken into account when choosing a test organism (Rand and Petrocelli 1985; Nussey 1994; Wepener 1997).

- since sensitivities vary among species, species with a broad range of sensitivities should be used whenever possible.
- must be a widely available and abundant species.
- species should be susceptible to routine maintenance in the laboratory and for culturing and rearing them in the laboratory so that chronic toxicity tests can be conducted.
- a test organism must be part of a food chain which can influence people or any other important species.
- in a biological community a test organism must show a low variability on genetic and niche level.
- a test organism must easily be identified because taxonomic indistinctness can influence data interpretation.
- an area's ecological balance cannot be disturbed when the test organism is removed form its natural habitat.

- test organisms must also be available throughout the year and not be too expensive.
- a sufficient number of test organisms of the same size and age must be available for tests.
- a test organism must be tested effortlessly, without using high-priced instruments and must not be labor-intensive.
- a test organism must accumulate pollutants rapidly thereby reflecting environmental levels of the pollutant, this facilitates a better understanding of their distribution.

Aquatic organisms like fishes, integrate all the stresses placed on the aquatic ecosystem. These organisms reflect the combined effects over periods of time. Fishes are a measure of environmental health, because everything that happens on the landscape goes into the rivers. For many years fishes have been valued as excellent indicators of water quality. Fishes have also been the most popular test organism because they are presumed to be the best understood organism in the aquatic environment (Roux D.J., 1994 Stein, J. E., et al., 1992; De La Torre et al., 2000). Because fishes are in direct contact with their surrounding environment, any change in the environment will be reflected as changes in physiological processes and survival (Fernando M.D. and E.A., Moliner 1991; Barton B.A., and G. K., Iwama 1991; Wilson R.W. and Taylor E.W., 1993; Wepener 1997). There are a lot of advantages in using fishes as an experimental animal.

- fish populations and individuals generally remain in the same area.

- fishes represent a wide range of tolerance from very sensitive to highly tolerant.
- the taxonomy of fishes is well established.
- fishes are easy to collect with the right equipment.
- lower cost and shorter developmental time.

Hence, in the present study *Channa punctatus* were chosen as test organism.

Fishery losses by pesticides

Many fish kills have been attributed to pesticides. In the 1960s and early 1970s, 18% of the reported fish kills were attributed to pesticides. Among major fish kills in which more than 100,000 fishes were killed, pesticides were the sixth ranking cause of death (responsible for 3.7% of the deaths). In aquatic systems pesticides cause fishery looses in several ways. These include high pesticide concentration in water, low level doses that may kill highly susceptible fishes, the elimination of essential fish food, or the reduction of dissolved oxygen level in the water due to the decomposition of aquatic animals and plants killed by pesticides. Each year large numbers of fishes are killed by pesticides based on EPA data, we calculated that from 1994-2004 the number of fish kills due to all factors was 145 million fish per year. (EPA 1994 - 2004). Studies have shown that high dosages of herbicides, insecticides, pesticides for weed and pest control are extremely toxic to fish (Corbett J.R., 1974; Jeyaratnam J. et al.,1982; Monsanto Company

1985; Cranmer M.F., 1986; Sawada Y., Nagai 1987; Asztalos et al., 1990; Tominack et al., 1991; Wesseling 1993; O'Malley M., 1997).

Purpose of physiological investigations:-

Fish live in very intimate contact with their environment and are therefore very susceptible to physiochemical changes, which may be reflected in their blood components. In fishes, exposure to chemical pollutants can induced either increase or decrease in haematological levels (Agrawal S.J., Srivastava A.K., 1980; Gill T.S. and Pant J.E., 1981; Hughes 1981; Sharma R.C. and Gupta N., 1984; Kori-Siakpere O., 1985; Pascoe et al., 1986; Kori-Siakpere 1991; Samprath et al., 1993; Annune P.A., et al., 1994a; Shastry K.V., et al., 1997; Neumosok J. G, Hughes G. M., 1998; Das et al., 2004). The count of red blood cell is a stable index and the fish body tries to maintain this count with in the limits for certain physiological standards using various physiological mechanism of compensation. Studies have shown that when the water quality is affected by contaminants, many physiological changes will be reflected in the values of haematological parameters (Haley 1979; Guzelian 1982; Suege J. and Bluzat R., 1983; Matsumura F., 1985; Hoar S.K, et al., 1986; Moses M., 1989; Tai T., et al., 1990; Edwards 1991; Talbot 1991, Mbiapo Youovop 1993). Blood cell responses are important indicators of changes in the internal and / or external environment of animals. The percentage of haemoglobin may also be affected by the toxicants. Any changes in blood parameters of fishes which occur because of the internal injuries can be used to determine the toxicity level of pesticides. (Fry F.E. 1971; Matthiessen P., 1981; Lakota et al.,1983; Golow A.A. and Godzi T. A. 1994; Alabaster J.S. and R., Lloyd 1982; Gaur et al.,

1992; Extoxnet 1995).

Stressors induce some changes that alter the homeostasis of the animals. The stressors first activate the chromaffin cells present in the walls of the cardinal veins and in some cases the heart and kidneys of the teleosts (Kleerekoper et al., 1973; Rand 1977; Mazeaund 1981; Natarajan 1981; Peakall 1992; Weber D.N., Spieler R.E. 1994; Dodson et al., 1995; Beauvais S.B., and Jones S.B. 1999; Beauvais S.L. et al., 2000; Brewer et al., 2001). Chromaffin cells release adrenaline and a small amount of nor adrenaline that stimulates the liver glycogen into blood glucose and the utilization of glucose by muscles. Blood sugar has a direct correlation to metabolism. The organs involved in maintaining the proper blood glucose levels is liver. Any changes caused by toxicants alter the homeostasis as affecting the blood parameters and functions of liver of the fishes (Hochachka 1978; Shastry K.V. and Siddiqui A. A. 1982; Ghosh 1987; Ghosh 1989; Jyothi B. and Natrajan G.M. 1999; Kumar Hemant and Gupta A.B. 1997).

The biggest sources of water in Bundelkhand region is Betwa river. Betwa rises from M.P. and meets the river Yamuna in Hamirpur district in U.P. The Betwa basin includes parts of a number of district in Bundelkhand region, like Sagar, Tikamgadh, Chattarpur, Lalitpur, Jhansi, Jalaun and Hamirpur. Except Betwa river Bundelkhand have rich source of other water reservoir as Cane river, Matateela dam and Pahunj river. These water reservoirs have become rich source of fish farming and valuable sources of fish food reaching to different areas of our country. The *Clarius batrachus, Leabio rohita, Heteropneustes fossiles* and *Channa punctatus* are some fishes mainly used for food.

These fishes are continuously challenged or stressed by lethal and sub lethal levels of contaminants. Although several studies have been carried out on physiological parameters exposed to different toxicants but still a little work has been done in this region. Fish haematology is used in recent to detect the stress caused by pesticides. Since glyphosate, phosphamidon, imidacloprid and metasystox are extensively used for pest and weed eradication in Bundelkhand region, it is necessary to study their hazardous effect on the aquatic system especially on fresh water fishes. Due to their food values *Channa punctatus* are in high demand. Thus the present investigations of haematological and biochemical parameters are undertaken to evaluate the effect of the (glyphosate, phosphamidon, imidacloprid and metasystox) toxicants in fresh water teleosts *Channa punctatus*.

Chapter - 2

REVIEW OF LITERATURE

REVIEW OF LITERATURE

The desire of innovation in man has contributed to pollution in the life and ecology of plants, fishes and other animals and microbes. Increased demand for food and other useful material has led to the chemicalization of agriculture and we have reached on such a stage that modern agriculture is dependent on high yielding varieties, which can only be grown under the influence of fertilizers and pesticides. Pesticides are the man made chemicals which are being used to produce enough cheap food. In India, 90,000 millions of technical grade pesticides are used annually to control pests and plant diseases. The steady flow of agricultural effluent discharge into water bodies increases the range of pollutants, which becomes evident when considering toxic pollution (Dhilon S. S., and Gupta A. K., 1983; Forstner U., and Prosi F 1989; Peakall D. 1992; Braunbeck T.,1994; Heath A. G. 1995; Mason C.F. 1996; Heath A. G. 1996; Das R. 2000; Santhakumar et al., 2000; Alam, M. N. and D. N. Sadhu 2001; Mishra et al., 2001; Lata S., et al., 2001; Singh S and D. N. Sadhu. 2001; Alam M. N., 2002; Patnail et al., 2002; Tilik 2005; Radha et al., 2005). Indiscriminate use of pesticides, careless handling, accidental spillage, or discharges of treated effluents into natural waterways have harmful effects on the fish population and other forms of aquatic life and may contribute long term effects in the environment. Water pollution is globally as a potential threat to both human and other animal populations (Verma S.R., et al 1990; Heath AG., 1995; Heath AG., 1996; Braunbeck, T., 1994; Mason C.F., 1996; Hrudey et al., 1996). Pesticides have been the focus of interest for environmentalist because of their wide spread use, transport, distribution in the

ecosystem. Chemicals with potential for wild life damage of aquatic life include heavy metals, organochlorine, organophosphate carbamate and cypermethrin. (Flos et al., 1987; Abidi R. and U.S., Srivastava 1988; Benerjee G., and Rajendranath 1990; Kumar B. and Banerji V., 1990; Kori-Siakpere O., 1991; Mishra B. K., 1993; Akira Kakuno and Jiro Koyama 1994; Bala et al., 1994; Martinez et al., 1994; Ahmad et al., 1995; Gupta et al., 1995; Khattak I. U. D., Hafeez, M. A. 1996; Kumar Hemant and Gupta A.B.,1997; Alkahem et al.,1998; Dethloff et al., 1999 Kori-Siakpere O., Egor V.E. 1999; Dhembare A.J., and Pondha G.M. 2000; Chandra et al., 2001; Aguigwo J.N. 2002; Sherry P. M., and Abidi Z. Z., 2002; Das et al., 2004; Kuruppasamy et al., 2005; Ajani F., 2008; Khalid et al., 2008). Reports of the presence of pesticides in water and food materials have aroused great concern among the scientists and bio researchers, hence led to studies on their toxic potential. There are numerous bioresearchers who gave the creative evidence of presence of pesticides in aquatic media (Bhatia, H.L. 1972; Verma, S.R., et al., 1979; Sehgal and M.K. Bhasin 1985; Jhon, P.J. Rathor. And A. Prakash 1989; Verma, S.R., et al 1990; Rajendranath,T. and G. Benerji.,1991; Jain, R. and K.D. Mishra. 1993; Sexena, V., et al., 1997; Das, R. 1998; Santhakumar, M., et al., 2000; Alam, M. N., 2002; Radha, G.S., et al., 2005).

Now a days organophosphorus pesticides are widely used to control the variety of agricultural pests (Khangarot B.S. and P.K. Ray 1987a; Mukherjee M.K. and S.K., Knor.1984; Mishra U.K. 1988; Ghosh and Chatterji 1989; Khangarot B.S., et al., 1996 Mishra et al., 1998; Tilik K.S., 2005; Trivedi, S. and D. N., Saksena 1999; Benerji G. and Rajendranath 1999; Dhembare A.J. and Pondha, G.M., 2000;

D. S. et al., 2001 Singh S. and D. N., Sadhu. 2001; Delor M.E., et al 2002). Vvarious types of phosphorus group are present in the major class of organophosphorus pesticides. Like as Phosphate group, *O* - alkyl phosphorothioate, phosphorodithioate, *S*-alkyl phosphorothioate, phosphoramidate etc. At least 100 organophosphorus pesticides have been reviewed by WHO for consideration as agents for the control of disease vectors. A large number have been reviewed by the FAO/WHO Joint Meetings on Pesticide Residues. Unlike many compounds scrutinized by the IPCS, these compounds are designed to be toxic for certain pests and are added deliberately to the environment (Gill et al.,1990; Benerjee G., and Rajendranath 1990; Balasubramanian S. and Ramaswami M. 1991; Gaur K Pandey Surendra D 1992; Chandrasekhar S and N., Natrajan 1993; Chaturvedi L.D., and Agrawal K., 1993; Chandrasekhar and S., Jayabalan 1993; Sadhu D.N., 1993; Bhattacharya Lata 1994; Gupta et al., 1995 ; Fryday et al., 1996; Kumar et al., 1997; Kumar Hemant and Gupta A.B., 1997; Jebakumar et al.. 1997; Benerji G., and Rajendranath 1999; Nath. Ravindra, Banerajee V. 1999; Armbrust K.L., 2000 Delor et al., 2000; Dhembare A.J. and Pondha G.M. 2000; Kamble G.B. and Muley D.V. 2000; Jhosi P. and Deep H. 2002; Medda et al., 2002; Noori et al., 2003; Ayoola S.O. 2008). Although the oraganophosphorus pesticides are used to control the insect, pests but they are harmful to other non target organism like fishes as they are continually added to water bodies. Several reports of the excessive use of organophosphorus pesticides and their toxicity on fishes and other aquatic animals are available (Charistoforides C., & Hedley-Whyte, J.1969; Blaxhall P. C.1972; Ranke B., and Rybicke 1975; Lone K..P. and M. Y. Javed; 1976 Ransfold K. D.1978; Agrawal S. J. and A. K.

Srivastava 1980; Pandey et al., 1980; Jagdish et al., 1981; Goel et al., 1982; Grues et al., 1983; Mishra J., Shrivastava A. K. 1983; Natarajan G.M. 1983; Kulshrestha S.K. and Arora A.1984; Jai Nath et al., 1984; Dange A.D., 1986; Khangarot B.S., and P.K. Ray 1988e; Ghosh T. K., 1989; Mahesh et al., 1989; Gill et al.,1990 Benerjee G., and Rajendranath 1990; Balasubramanian S. and Ramaswami M. 1991; Gaur K Pandey Surendra D 1992; Chandrasekhar S and N., Natrajan 1993; Chaturvedi L.D., and Agrawal K., 1993; Gupta 1995; Jain R and K.D. Mishra. 1995; Jeba kumar S.R.D. et al., 1997; Mishra 1997; Sastry N.N., et al., 1997; Khangarot et al., 1996; Poonmani R. and B. Dhanakkodi. 1996; Kumar et al., 1997; Kumar Hemant and Gupta A.B., 1997; Jebakumar et al.. 1997; Imbamani, N. and R., Shrivassan 1998; NeumosoJk.G, Hughes G. M., 1998; Benerji G., and Rajendranath 1999; Das R., 2000; Dhembare A.J. and Pondha G.M. 2000; Santhakumar et al., 2000; Alam, M. N. and D. N. Sadhu 2001; Mishra D. S. et al., 2001; Lata S., et al., 2001; Singh S and D. N. Sadhu. 2001; Alam M. N., 2002; Delor et al., 2002; Patnail et al., 2002; Tilik 2005; Radha et al., 2005).

Herbicides are also widely used for the control of water plants and weeds which may impede the flow of water during the summer; when sudden heavy rain can cause flooding (Annune et al., 1994). Harmful herbicides enter surface water with the discharge of agricultural wastes. It is biologically very reactive and hence gives rise to both acute and chronic poisoning toxic effects of pesticides on aquatic organism like fishes. Several workers have investigated the physiological disturbance caused by herbicides exposure on fishes (Brooker M. P. and Edwards R. W. 1975; Monsanto Company 1985;

Hoar et al., 1986; Mitchell et al., 1987; Pulla et al., 1987; Sawada Y, Nagai Y., 1987; Mishra U.K. 1988; Moses M., 1989; Anju Kumari and K., Pandey 1990; Tai T, et al., 1990; Talbot et al., 1991; Tominack, et al., 1991; Neskovic et al., 1996; Mishra 1997; Cox C. 1998; Mishra et al., 1998; USDA 1984). Numerous workers have investigated the disturbance in carbohydrate metabolism caused by various toxicants exposure on fishes (Hochachka, P.W., 1978; Srivastava A. K.,1981; Shrivastva A. K. and Singh N. N.,1981; Shastry K.V. and Siddiqui A. A. 1982; Singh H. H., Srivastava A. K., 1982; Shaikha Y.A. and P.K., Hiradher 1985; Ghosh T. K., 1987; Ghosh T. K. 1989; Shobha et al., 1989; Kumar Hemant and Gupta A.B.1997; Odiete W.O.1999; Jyothi B. and Natrajan G. M., 1999).

Aquatic toxicologists traditionally have been interested in determining how much of the toxic chemicals, fishes (or other aquatic creators) can tolerate before they die. This is done by standardizing the tolerable concentrations of pesticides during acute toxicity experiments. One of the purpose of the toxicity studies is to conclude whether a potential toxicant harmful to aquatic life, and if so, to find out the relationship between toxicant and concentration and its consequence on aquatic animals (especially fishes). In this manner various bioresearches have been investigated the acute toxicity effects of pesticides, insecticides and herbicides on aquatic animals (Pandey et al., 1980; Jagdish et al., 1981; Goel et al., 1982; Grues et al., 1983; Mishra J., Shrivastava A. K. 1983; Natarajan G.M. 1983; Kulshrestha S.K. and Arora A.1984; Jai Nath et al., 1984; Dange A.D., 1986; Khangarot B.S., and P.K. Ray 1988e; Ghosh T. K., 1989; Mahesh et al., 1989; Gill et al.,1990 Benerjee G., and Rajendranath 1990;

Balasubramanian S. and Ramaswami M. 1991; Gaur K Pandey Surendra D 1992; Chandrasekhar S and N., Natrajan 1993; Chaturvedi L.D., and Agrawal K., 1993; Chandrasekhar and S., Jayabalan 1993; Sadhu D.N., 1993; Nath. Ravindra, Banerajee V. 1999; Armbrust K.L., 2000 Delor et al., 2000; Dhembare A.J. and Pondha G.M. 2000; Imbamani N., and R Shrivassan 1998; Kamble G.B. and Muley D.V. 2000; Jhosi P. and Deep H. 2002; Medda et al., 2002; Noori et al., 2003; Ayoola S.O. 2008).

The suitable biomarker tests that cover all the biological activities and functions of the organism and that can make it possible to judge which of the functions were abnormal, when the organism has been exposed to toxicants These are blood parameters which considered pathophysiological biomarkers of the whole body (Silbergeld E.K., 1974; Adams S.M., 1990; Peakall D. 1992; Stein, J. E., et al., 1992; De La Torre et al., 2000; Vander et al., 2003). Several works have been carried out on heamatological and biochemical parameters to determine the effected biological activities on test organism because the blood parameters are the best biomarkers to investigate the hazardous effect of pollution cause by various toxicants (Bhatia H.L.1972; Blaxhall P. C. 1972; Mahajan C. L., and Juneja S. 1979; Pandey et al., 1980; Jagdish Mishra and Anil K. Shrivastava 1981; Sastry K.V and K. Sharma 1981; Goel et al., 1982; Bhaskar B.R., and K.S. Rao 1985; Kori-Siakpere O. 1985; Casillas, E. and L.S Smith 1987; Sastry et al., 1988; Mahesh et al., 1989; Birendra Kumar and Banerjee V. 1991; Kori-Siakpere O. 1991; Sastry K.V. and A. Gupta 1994; Gupta et al., 1995; Khattak I. U. D., Hafeez, M. A. 1996; Kori-Siakpere O, Egor V.E. 1999; Nath. Ravindra, Banerajee V.

1999; Dhembare A.J., and Pondha G.M. 2000; Das B. K. and Mukherjee S.C. 2000; Chandra et al., 2001; Jhosi P. and Deep H. 2002; Sherry P. M., and Abidi Z.Z. 2002 Srivastava R.K. and Srivastava S. 2002; Das et al., 2004; Khalid et al., 2008; Ramesh M., and Saravanan M. 2008; Saufy et al., 2007).

Glyphosate, Phosphamidon, Metasystox and Imidacloprid are four toxicants studied in this toxicological study because they are widely used in all over world and Bundelkhand region, but very scanty informations are available regarding Glyphosate, and Imidacloprid intoxication of fish haematology. Several workers have been carried out in phosphamidon and metasystox intoxicated fishes in India except Bundelkhand region. (Comes et al., 1976a; Rueppel et al., 1977; Roy et al., 1977; Folmar et al., 1979; Mahajan and Juneja 1979; Sullivan, T. P., and D. S. Sullivan. 1979; Hildebrand et al., 1980; Roslycky E. B. 1982; Natrajan G.M., 1983; Morrison et al., 1984; Newton M. et.al. 1984; Monsanto and Company 1981; Monsanto Company, 1985; Hildebrand et al., 1986; Mitchell et al., 1987; Servizi et al., 1987; Jhon P.J., Rathor. And A. Prakash 1989 ; Roy et al., 1989b; Santillo et al., 1989a; Anton et al. 1993; E.P.A. 1993; Liu et al., 1993; Marrs, et al., 1993; Roberts R.O. and S.G. Berk. 1993; Minaxi Das and Shambhu Prasad 1994; Giesy 2000; Feng et al., 1990; Nagata 1996; Neskovic. et.al. 1996; Shekhar P., and I., Christy 1996; Matsuda et al., 1998; Linz et al., 1999; Tomizawa M. and Casida J. E. 2000; Williams et al., 2000; Anand Kumar 2001; Sheetset al., 2001; Josif Jhon P. 2007).

Aannd kumar et al., (2001) reported decreased level of Hb%, TEC and PCV after both acute and chronic exposure to phosphamidon

on *Heteropneustes fossilies*.

Ravindra Nath and V., Vanerjee (1999) reported decreased TEC, Hb% and PCV% but TLC was increased significantly in lethal and sub lethal toxicity of rogar pesticide with in 24, 96 hours and 7 days exposure. Anju kumari and A.pandey (1990) reported the decrease in Hb content TEC and PCV in *Clarius batrachus* intoxication of teficide and butachor follow acute toxicity of 48 hours according to Chandshekhar S. and N., Jayabalan (1993) Hb % and PCV decreased after exposed to sublethal concentration of endosulphan for different period used like as 7, 14 and 21days. The decrease level in Hb %, TEC, PCV and increase in TLC were reported by several workers using oreganophosphorus pesticides and other toxicant in acute toxicity bioassay experiment in fishes (Mukhopadhyay and Dahadrai 1980; Jagdish Mishra and Anil K. Shrivastava 1981; Kori-Siakpere 1985; Chakraporti P. 1986; Homechaudhuri 1986; Anju kumari and A.pandey 1990; Mishra B. K. 1993; Nath. Ravindra and Banerajee V., 1999; Kori et al., 2008; Ajani F. 2008). Similar responses of Hb %, TEC and PCV were also observed by many authors in chronic cases using oreganophosphorus pesticides and other toxicants in fishes (Sastry K.V. and K., Sharma 1982; Natrajan G.M. 1983; Pandey 1984; Shastry et al., 1984; Shastry et al., 1984; Gill T.S. and Pant J.C. 1985; Rajeshwari et al., 1989; Mala and Shreak 1990; Ruparelia et al., 1991; Patel S.K. and P.G. Parmer 1993; Chandshekhar S. and N. Jayabalan 1993; Mughal et al., 1993; Verma G.P. and Pranamita Panighari 1998; Alkaham et al., 1998; Kori-Siakpere et al., 1999; Nath. Ravindra, Banerajee V. 1999; Anand Kumar 2001; Muhammad Atamanalp and Telat Yanik 2002;

Nuri at al., 2003 Atef M. Al. Atter 2005; Vutukuru S.S. 2005; Shah S.L., 2006; Velisek et al 2006 a, b; Ajani F., 2008; Khalid et al., 2008; Ramesh M. and Saravanan M. 2008; Khalid et al., 2008).

The increase level of TLC was also observed by several bioresearchers using various toxicants (Shastry K.V and K. Sharma 1982; Pandey 1984; Shastry et al., 1984; Gill T.S. and Pant J.C. 1985; Kori-Siakpere O. 1985; Chakraporti P.1986; Homechaudhuri 1986; Bielinska 1987; Rajeshwari et al., 1989; Ruparelia et al., 1991; Thakur G. K. and P. K. Pandey 1991; Chandshekhar S. and N. Jayabalan 1993; Mishra B. K. 1993; Mughal et al., 1993; Patel S.K. and P.G. Parmer 1993; Kori-Siakpere 1999; Nath. Ravindra, Banerajee V. 1999; Anand Kumar 2001; Muhammad Atamanalp and Telat Yanik 2002; Atef M. Al. Atter 2005; Shah S.L. 2006; Ramesh M. and Saravanan M. 2008).

Minaxi das and Shambhu Prasad (1994) reported increase level of TEC (total erythrocytes count) and Hb% (haemoglobin percentage) using metasystox during 48 hours exposure on *Heteropneustes fossilies*. Increased parameters like as TEC and Hb% which originate in this case have also been reported earlier by Mahajan and Juneja 1979; Dhilon and Gupta 1983 ; Mishra and Shrivastava 1985; Ghosh and Chaterjee; 1989 during acute toxic exposure. G.M. Natrajan (1983) also reported the increase in total RBC count, PCV, and Hb% in *Heteropneustes fossilies* following chronic toxicity with 30 days exposure of metasystox. Similar results of haematological effects on fresh water fishes using different pesticides have also been established prior (Valicre E.J., and Stickhey C.J., 1999; Kumar B.K., 1991; Lavin 1992; Kuruppasamy et al., 2005). Few workers are reported the

decrease level of TLC into toxicants on fishes following exposure investigation Muhammad Atamanalp and Telat Yanik 2002 Verma G.P. and Pranamita Panighari 1998; Khalid et al., 2008 Maule A.G., Schreck C.B. 1990.

Malla et al., (2009) reported increased value of ESR after acute exposure of 24, 48, 72, and 96 hours intoxication of chloropyrifos on *Channa punctatus*. Similar reports have also been done by copious bio-researchers using different toxicant in both acute and chronic toxicity (Kumar B., and Banerjee V. 1990; Singh S., and Bhati DPS. 1991; Chaturvedi L.D., and Agrawal K.1993; Bala Sashi et al., 1994; Goel K.A., and Maya 1996; Nath. Ravindra and Banerajee V. 1999). Nuri at al., (2003) reported the MCHC decreased with increase cypermethrin concentrations, but MCV level increased and MCH was not affected with exposure of deferent cypermethrin concentrations (Van Vuren, J.H.J. 1986). The various alterations in values of MCH, MCHC and MCV were also carried out during acute and chronic toxicity bioassay investigated by many workers (Verma et al., 1979; Jagdish Mishra and Anil K. Shrivastava 1981; Natrajan G.M. 1983; Pandey 1984; Gill and Pant 1985; Kori-Siakpere O. 1985; Chakraporti P. 1986; Homechaudhuri 1986; Bielinska 1987 Ramawamy M. and G.T. Reddy 1988; Bradury and Coats 1989; Rajeshwari et al., 1989; Anju kumari and A.pandey 1990; Mala and Shreak 1990; Singh H.S. and Reddy T.V. 1990; Ruparelia et al., 1991; Mishra B. K., 1993; Patel S.K. and P.G. Parmer 1993; Alkaham et al., 1998;Verma G.P. and Pranamita Panighari 1998; Sopinska and Guz 1998; Kori-Siakpere 1999; Nath Ravindra, Banerajee V. 1999; Das and Mukherjee 2000; Muhammad Atamanalp and Telat Yanik 2002; Nuri et al., 2003;

Svobodova et al., 2003; Atef M. Al. Atter 2005; Prashanth et al., 2005; Vutukuru S.S. 2005; Dobsikova et al., 2006; Velisek et al 2006 a;b 2007; Ajani F. 2008; Khalid et al., 2008; Velisek et al., 2009).

Kumar Hemant and A.B., Gupta (1997) reported the increased level of glucose after acute and chronic exposure of glyphosate and metasystox in *Heteropneustes fossilies* (Verma G.P. and Pranamita Panighari 1998; Khalid et al., 2008).

Chandrasekhar S. and N., Natrajan (1993) reported the increase in blood glucose in fishes exposed to endosulfan in *Heteropneustes fossilies*. Other workers also reported hyperglycemic condition in fishes. (Srivastava A.K., 1981; Nemcsok and Bones 1982; Singh H.H., Srivastava A. K. 1982; Ghosh, T. K. ,1989; Nataragan G. M., 1989; Medda et al., 1993; Kumar Hemant and A.B., Gupta 1997; Jyothi B. and G. Narayan 1999; Luskova et al., 2002; Saufy H., et al., 2007).

Temperature of aquatic environment is important for ensuring survival, distribution and normal metabolism of fishes (Forghally et al., 1973). O_2 level, Salinity or other environmental factors also caused disturbance in hormones, metabolic pathways, enzymes and behaviors of fishes (Gubbins et al., 2000). Haematological parameters are also valuable tools for monitoring the health of fishes and they are affected by many endogenous and exogenous factors (Coutant C.C., 1972; Connors et al., 1978; Wilson R.W. and Taylor, E.W. 1993; Howerton R., 2001).

Numerous bioresearchers have been reported the seasonal variations on blood parameters in fishes and other animals (Charistoforides, C. & Hedley-Whyte, J., 1969; Bridges, D. W., et al.,

1976; Cech, J.J. and D.E. Wohlschlag, 1981; Cech, J.J. and D.E. Wohlschlag, 1981; Raizada, M.N., K.K Jain and S. Raizada, 1983; Harding, J and Hogland, L. B., 1984; Joshi, P.C., 1989; Denton, J.E. and M.K., Yousef, 1995; Collazos, M.E., et al., 1998).

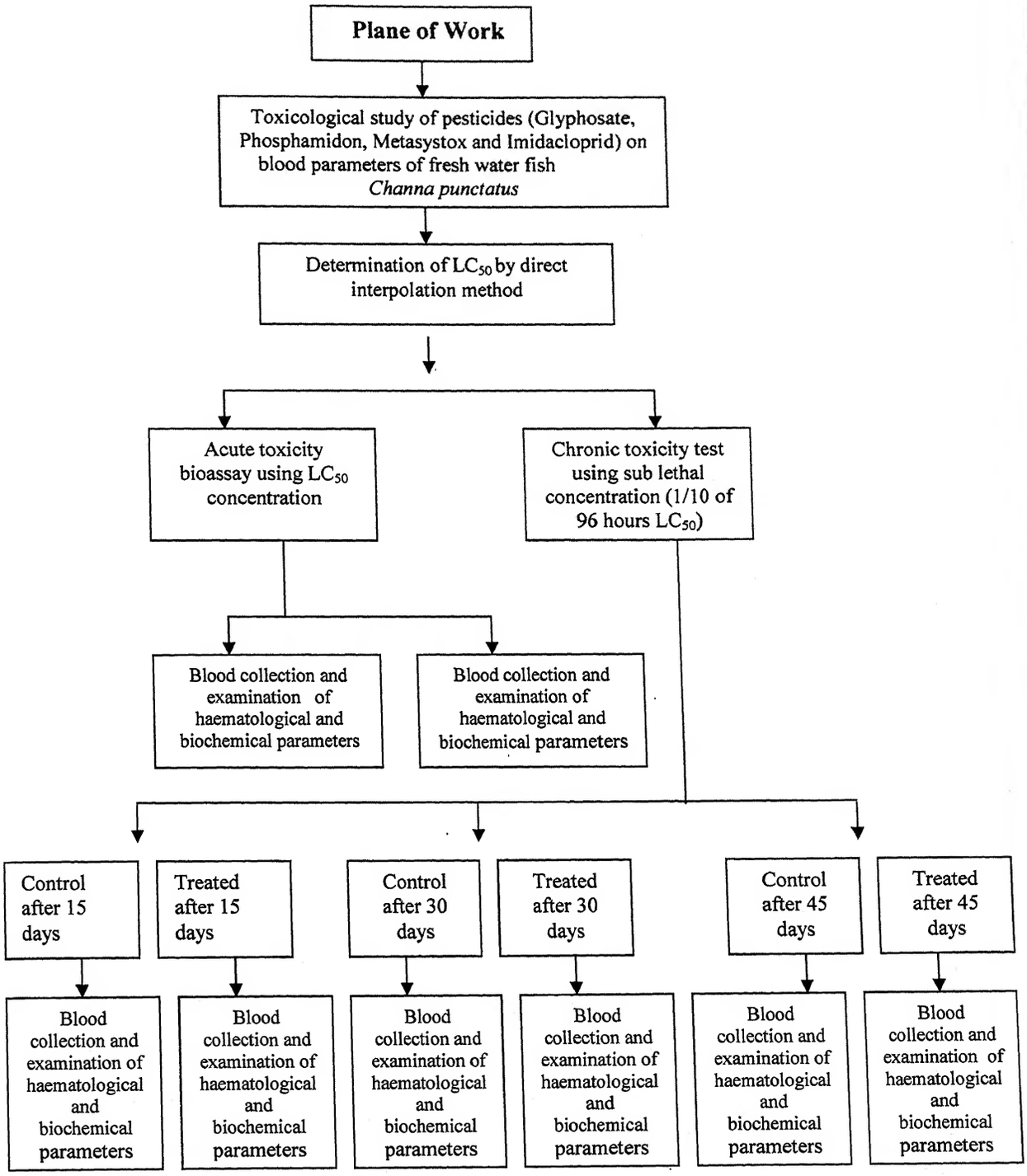
Chapter - 3

EXPERIMENTAL DESIGN AND METHODOLOGY

EXPERIMENTAL DESIGN AND METHODOLOGY

Toxicity tests are experiments or trials designed to assess or evaluate the concentration of toxicants and the duration of exposure required to produce a criterion effect. The aquatic toxicity tests are frequently referred to as bioassays. In order to examine the various effects associated with various length of exposure, the ideal and most widely accepted testing frame work includes acute, sub acute and long term toxicity tests. The acute and sub acute toxicity tests are time bound experiments and these tests were carried out in the present investigation to evaluate the response of the toxicants. The term acute can be used to define either the exposure or the response with in short duration exposure. The results of acute toxicity test are represent in terms of LC_{50} (median lethal concentration) for aquatic species. The LC_{50} value represents the ppm or mg/l concentration of the toxicant that would kill 50% of population of a particular aquatic species. The LC_{50} is the usual method of reporting acute toxicity results. The acute toxicity data are important and beneficial in the fixation of sub lethal concentration for chronic toxicity tests.

Sub acute toxicity is also known as chronic toxicity. This study involves a stimulus which is less severe than the acute stimulus and produces a response in longer time and may become chronic. In this the fishes are exposed to chemicals at levels much lower than those that are acutely fatal but they are exposed over a longer period of time. To assess the native of the toxic effects in more realistic situations sub acute toxicity test were conducted.



*All the acute and chronic toxicity experiments were carried out during winter, summer and rainy seasons. Comparisons of different haematological and bio chemical parameters were presented graphically among different season.

Acute toxicity tests

A. Collection of water sample: -

Chlorine free tap water was used through out the course of the experiment. The physiological characters of water sample like the temperature of the test medium, dissolve oxygen, alkinity, hardness and specific conductivity were tested in the Zonal laboratory U.P. Jal Nigam Babina, Jhansi.

B. Collection of fish Sample: -

During the whole toxicological investigation the fishes *Channa punctatus* were used. These are carnivorous fishes feeding upon insects crustaceans and invertebrates. These fishes are supposed to be good, nutritive fish and easily available in living condition through out the year and are good models for experimental work. The fishes were collected from different water bodies of Bundelkhand region with the help of professional fisher man. Live and healthy fishes were used in all the toxicological investigations. The selected fishes were checked against injury, infection or diseases by keeping in 0.2% of potassium permanganate solution for 1-2 minutes. They were kept in glass aquaria having a capacity of more than 40 liters. All over the experiment water was changed daily. The fishes were acclimatized in laboratory condition for 6-10 days. During acclimatization the fishes were fed egg albumin, earth worms and small insects.

C. Preparation of toxicants: -

The four toxicants namely glyphosate, phosphamidon, metasystox, and imidacloprid were selected in the present experiment

which are used in Bundelkhand region. Their trade name, chemical formula, emulsifiable / soluble concentrations and manufacture's name are given in chapter 4. The solution of toxicant was prepared by dissolving the toxicant in 100 ml of distilled water. From this stock solution desired concentrations of each toxicants were added in test medium. To determine the LC_{50} value several concentrations of toxicants were prepared separately. For example for the preparation of 0.025 ml/liter first we dissolve 2.5 ml of toxicant in 100 ml of distilled water. Then we add 1 ml of that solution in one liter of test medium. If the jar contains 5 liter tap water we add 5 ml of stock solution now concentration will be 0.025 ml/liter.

D. determination of LC_{50} value: -

After acclimatization feeding was stopped 24 hrs prior and during the exposure period. For the accurate determination of LC_{50} values two exploratory and one definitive test were conducted. During initial exploratory examination two concentrations were preferred to guess supposed mortality between zero percent to 100 percent. The two concentrations (lower and higher) of toxicants were introduced in two different jars containing five fishes each. In next (second) exploratory or range finding test 4 or 5 concentrations were taken to get narrow range of concentration for LC_{50} determination. Four or five fishes were exposed against each concentration. From the derivation of second range finding examination several (7-9) different concentrations were preferred for definitive test and ten fishes were exposed to each concentration. The mortality was recorded after a period of 24, 48, 72 and 96 hrs and dead fishes were removed when observed. The concentrations from the definitive test were employed

to determine the LC_{50} values by plotting a dose response curve between percent mortality and concentrations of toxicants (Direct interpolation method). A line was drawn between the point represent the % mortality and concentrations. The concentrations at which this line crosses the 50% lethality line was the actual lethal concentration of toxicant.

E. Collection of blood sample for Haematological & Biochemical parameters:-

In order to find out the effect of each toxicant for haematological and biochemical parameters blood was collected from the fishes of treated and control groups. The experimental plane is given below -

For acute toxicity test

After calculating the LC_{50} values 40 healthy fishes from different water bodies were collected, acclimatized and divided into five groups (A, B, C, D, and E) of eight fishes each. The LC_{50} concentrations of toxicants at 96, 72, 48, 24 hours were added to group D, C, B and A respectively. The 5th group maintained in water without toxicants served as control group. After completing the acute toxicity experiments blood was collected in vials, from each group by severing the caudal peduncle. Small quantity of blood was transferred in oxalated vials and remaining blood was centrifuged to separate the serum. Blood parameters were measured by the method described later.

For sub acute toxicity test: -

For chronic toxicity test 60 fishes were collected from market and washed with 0.2% $KMnO_4$ to avoid any dermal infection and acclimatized at least 10 days in laboratory condition. The fishes were divided into two groups of 30 fishes each. Group one was exposed to sub lethal concentration (1/10 of 96 hours LC_{50}) of toxicants. The second group was kept as untreated control. After 15, 30 and 45 days 10 fishes from each group were sacrificed, blood was collected and serum was separated for the testing of blood parameters.

Methodology

Preparation of oxalated vials:-

1. Ammonium oxalate 0.8gms
2. Potassium oxalate 1.2gms
3. Natural formalin 0.1ml
4. Distilled water 100ml

Mix both type of oxalates and add 0.1 ml of natural formalin. After ward transfer this mixture into 100 ml of distilled water. Hence prepared solution was poured in vials and incubate for 10 minutes. Consequently oxalated vials are ready for the use of blood collection.

Estimation of haemoglobin

Apparatus: -

Haemoglobinometer consisting of two comparator with glass standards, a cylindrical Hb tube marked both gram and percentage figures and Hb pipette marked at 20 cumm, 0.1N HCl and distilled

water.

Principle: -

Hb is converted into acid haematin by HCl. The brown colour of the compound is matched against a brown glass standard in a comparator.

Procedure: -

1. Fill the Hb pipette with non oxalated blood exactly up to 20 cumm mark by sucking. If a slight excess is drawn in it may be removed by touching the point of the pipette with the finger or gauge. Hb pipette or capillary should be filled carefully with out any bubbles.

2. Empty the pipette into graduated tube already filled by HCl up to the bottom graduation line (mark 2). Rinse the pipette at least three times by drawing in and discharging the blood acid mixture.

3. Mix the acid haematin solution in the tube with the glass rod and allow the tube to stand for ten minutes to develop the colour of the acid haematin.

4. Now dilute the solution of acid haematin by adding distilled water drop by drop, stirring the mixture with glass rod. The comparator is held against good day light and addition of water continued till the colour of the solution matches perfectly with that of the standards. Take the reading in grams percent. The bottom of the meniscus is read.

Total erythrocytes count (TEC):-

Apparatus: -

Red cell pipette, diluting fluid (Hayem's solution), Neubauer's counting chamber with cover slip (haemocytometer), Microscope etc.

Principle: -

Blood is diluted exactly 1:200 with a special pipette using an isotonic diluted fluid (Hayem's solution) for the preservation of the corpuscles. This diluted blood is placed in special RBC counting chamber (Neubauer's chamber). The cells are counted. The number of cells is multiplied by the appropriate factor to obtain the number of erythrocytes in 1 cu mm of undiluted blood.

Preparation of Hayem's diluting solution: -

This solution has following ingredients.

1. Sodium chloride	0.5gms
2. Sodium phosphate	2.5gms
3. Mercuric chloride	0.25ml
4. Distilled water	100ml

Mix well both type of salts sodium chloride and sodium phosphate and 0.25 ml mercuric chloride and the prepared combination was poured in 100 ml of distilled water.

Procedure:

1. Take the oxalated blood exactly up to the 0.5 mark in a special pipette (RBC pipette). Immediately draw up diluting fluid up to

the mark 101 by holding the pipette horizontally, thus making a dilution of 1:200 in pipette. Now rotating the pipette between the thumb and forefinger.

2. The cover slip is placed over the Neubauer's chamber so as to cover both the ruled platforms evenly.

3. Before charging the counting chambers liberate few drops of the diluted blood by the pipette and put a small drop at the periphery of cover glass which is already in position. Care must be taken so that the suspension does not flow into the moats on either side nor should be bubble remain beneath the cover glass. Allow 2-3 minutes to pass and check the red cells settle down properly.

4. Count the cells in 80 small squares. These 80 small squares comprise 5 medium size squares, each of which is bounded by a triple line. In counting, the cells which touch the left hand side lines or the upper lines of the square are taken to be with in that square and those which touch the lower or right hand side lines are omitted as out side the square.

5. The total volume of 80 small squares is 1/50 cu mm. The dilution is 1 in 200 so the RBC count is

$$\text{Dilution} \times 1/\text{volume} \times \text{No. of cells counted (N)}$$

$$= 200 \times 50 \times N$$

$$= 10000 \times N \text{ cells / cu mm.}$$

Total leucocytes count (TLC):-

Apparatus: -

White cell pipette, diluting fluid (Truck's solution), Neubauer's counting chamber with cover slip (haemocytometer), Microscope etc.

Principle: -

Blood is diluted exactly 1:20 with a special pipette using an isotonic solution. The diluted fluid truck's was used for preservation of the corpuscles. The diluted blood is placed in a special counting chamber (Neubauer's Counting Chamber) and the cells in a measured volume are counted. This figure is multiplied by the appropriate factor to obtain the number of leucocytes.

Preparation of Truck's solution: -

This solution has following ingredients.

1. Glacial acetic acid	3 ml
2. Gentian violet	1.0 ml
3. Distilled water	97ml

Mix glacial acetic acid in 97 ml of distilled water than add gentian violet.

Procedure:-

1. Draw oxalated blood in a clean dry pipette up to the 0.5 mark. Suck the WBC diluting fluid up to the mark 11. Mix it thoroughly and charge the counting chamber as described earlier. Allow 2-3 minutes to pass and check that all the cells settle down properly.

2. Count the white blood cells in the 4 corners of the chamber. Each of the corners (single ruled square) is divided into 16 smaller squares. Each four large corner squares (16 smaller squares) has an area of 1 sq mm. In counting the cells which touch the left hand side lines or the upper lines of the squares are taken to be with in that square and those which touch the lower and right side are omitted as out side the square.

3. The volume of 4 corner squares is 0.4 cu mm, Dilution 20, Number of cells N then,

$$1\text{cu.mm contain} = \frac{N \times 20}{0.4} = N \times 50$$

Erythrocytes sedimentation rate (ESR):-

Apparatus: -

Wintrobe tube, (Length 110 mm, diameter 3.0 mm, graduation of lower 100 mm. from 0-100) anticoagulant (double oxalate)

Principle: -

If the anticoagulant is added to the blood and the specimen allowed to stand in a tube, red cells slowly sediment to the bottom of the tube leaving clear plasma as the supernatant. The rate of sedimentation estimated under standard conditions is known as the Erythrocytes sedimentation rate.

Procedure:-

The tube is filled up to the 100 mm mark, allowed to stand in a

vertical position at room temperature for one hour and read the fall of the red cells.

Packed cells volume (PCV):-

Apparatus: -

Wintrobe tube, (Length 110 mm, diameter 3.0 mm, graduation of 100 mm. (from 0-100) anticoagulated blood, Centrifuge machine etc.

Principle: -

An oxalated sample of blood is centrifuged to pack red cells to the maximum. The volume of packed cells is determined. The procedure is reliable because its reproducibility.

Procedure:-

1. The wintrobe tube must be clean and dry. Mix the oxalated sample of blood thoroughly for 3 minutes. Fill the blood in the wintrobe tube up to the 100 division marks. There must be no air bubbles.

2. Fill a second Wintrobe tube with either another sample of blood or with water. This tube is to counter balance the first one during centrifugation.

3. Put the tube in the centrifuge and centrifuge it at 3000 r.p.m. for 30 minutes. Take the reading of the packed red cells. The original column of blood in the tube being 100 mm. The volume of packed red cells can be read directly as a percentage.

Mean Corpuscular Haemoglobin (MCH): -

For estimating the average haemoglobin content of a single red cell in microgram following formula was applied.

$$MCH = \frac{\text{Haemoglobin gm/100ml}}{\text{RBC in millions/Cubic millimeter}} \times 10$$

Mean Corpuscular haemoglobin concentration (MCHC): -

To find out the average haemoglobin percentage following formula can be used.

$$MCHC = \frac{\text{Haemoglobin in gm/100 ml}}{\text{PCV}} \times 100$$

Mean Corpuscular Volume (MCV): -

The formula for counting the MCV was

$$MCV = \frac{\text{PCV of /100 ml blood}}{\text{RBC in millions/Cubic millimeter}} \times 10$$

Blood Glucose:-

Apparatus: -

Autospan diagnostic kit, colorimeter, and distilled water. The kit contains the following reagents.

Reagent 1 (Glucose oxidase, peroxidase, 4-aminoantipyrine, buffer, stabilizers)

Reagent 2 (Diluent Phenol preservative)

Reagent 3 (Dextrose, benzoic acid)

To prepare the working glucose reagent quantitatively, transfer the contents of vial of reagent one and vial of reagent two in a clean black coloured plastic bottle.

Principle: -

Determination of blood sugar is one of the first biochemical estimation to be applied clinically. The abnormalities of blood glucose levels reflect disturbance in physiology.

Glucose is oxidized by glucose oxidase to gluconic acid and hydrogen peroxide. In a subsequent peroxidase catalyzed reaction, the oxygen librated is accepted by the chromogen system to give a red colored quinoneimine compound. The red colour so developed is measured at 540 nm and is directly proportional to glucose concentration (Silbergeld E.K., 1974; Folin and Wu., 1996).

Procedure: -

We take 3 test tubes and marked it B, S, T respectively. Afterward 20 µl of serum was taken in test tube marked T and 20 µl of glucose standard (reagent 3) is added in standard test tube marked S and third tube is kept as blanked marked B. Then add 1.5 ml of working glucose reagent (prepared by mixing reagent 1 and reagent 2

from the kit) into all three test tubes and then incubate them at 37°C for 10 minutes. To stop the reaction add 1.5 ml of distilled water into all three test tubes. Now we measure the absorbance (optical density) of the test and the standard against the blank at 540 nm.

Calculation: -

$$\text{Glucose (mg/dl)} = \frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \text{x100}$$

Statistical analysis:-

The statistical significance of data was determined by student' t test as heterogeneity of variance was noticed.

Chapter -4

CHEMISTRY OF TOXICANTS

(a.) Chemistry of Toxicants

Glyphosate

Glyphosate is a systemic herbicide that can control most annual and perinnial plants. It controls weeds by inhibiting the synthesis of aromatic amino acids necessary for protein formation in susceptible plants. This compound was first synthesized in 1955 by E. Beriger of CIBA limited Basle, Switzerland. Glyphosate is strongly adsorbed to soil particles, which prevents it from excessive leaching or from being taken-up from the soil by non-target plants. It is degraded primarily by microbial metabolism, but strong adsorption to soil can inhibit microbial metabolism and slow degradation. Photo- and chemical degradation are not significant in the dissipation of Glyphosate from soils. The half-life of Glyphosate ranges from several weeks to years, but averages two months. In water, Glyphosate is rapidly dissipated through adsorption to suspended and bottom sediments, and has a half-life of 12 days to ten weeks. Glyphosate by itself is of relatively higher toxicity to fishes and other aquatic organisms.

Glyphosate, also known by the trade names Roundup for agricultural use, is a broad-spectrum translocated herbicide, used primarily in agricultural applications and for vegetation control in non-crop areas. It is used for aquatic weed control in fish-ponds, lakes, canals, slows running water, etc. (USDA 1984). Formulations of glyphosate include roundup have been extensively investigated for their potential to produce adverse effects in non-target organisms. Glyphosate is soluble in water, and tends to bind tightly to sediment, suspended particulates, organic matter and soil, becoming essentially

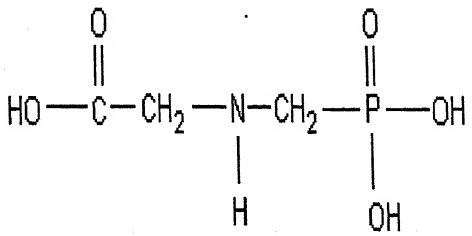
unavailable to plants or other aquatic organisms. Since glyphosate developed in the 1970's, there have been much documented cases of adverse effects on fishes and aquatic invertebrates associated its use for the control of aquatic weeds (Giesy et al. 2000).

Glyphosate is perhaps the most important herbicide ever developed. Literature of toxicological and ecotoxicological properties of glyphosate is extremely sparse, considering its importance as herbicide. Generally, glyphosate is slightly toxic to plant, but it may have an impact on the aquatic environment and also on the other aquatic organisms especially on fishes life. Due to this, its toxicity investigation is very important. The study of lethal and sublethal effects is of special importance for toxicological evaluation of compound. It occurs in two isomeric forms α and β in the ratio of 3:7. Its insecticidal property is mostly due to α isomer. It is used as systemic and also as stomach poison.

IUPAC name: N-(phosphonomethyl) glycine

Molecular formula: $C_3H_8NO_5P$

Structural formula:



Its trade names are mostly Roundup^R Armada; Kleenup^R (isopropyl ammonium); Spasor^R; Squadron etc.

Physical Properties: -

Pure Glyphosate is a colourless, odourless, crystalline solid with a melting point of 185 °C and decomposes at 187 °C producing toxic fumes including nitrogen oxides and phosphorus oxides. Solutions of the Glyphosate salts are corrosive to iron or galvanized steel. Pure Glyphosate is slightly soluble in water (12 g/litre at 25 °C), and is practically insoluble in most organic solvents. The alkali-metal and amine salts are readily soluble in water.

Mode of action: -

Glyphosate penetrates the plant leaf cuticle shortly after contact and begins a cell by cell migration to the phloem, from which it is transported throughout the plants. The herbicidal action usually occurs within 7 days and up to 30 for woody plants (Mc Laren and Hart 1995; Monsanto, 1985) Glyphosate's primary herbicidal mode of action is to block the synthesis of aromatic amino acids and the metabolism of phenolic compounds by disrupting the plant's shikimic acid metabolic pathway, leading to the inability of the plant to synthesize protein and produce new plant tissue. This is the only herbicide known to interfere with this particular pathway (McLaren and Hart 1995). A secondary mode of action affects the photosynthetic process, respiration and synthesis of nucleic acids by interacting with a complex series of enzymes which control synthesis of important molecules such as chlorophyll.

Phosphamidon

Phosphamidon is one of the versatile oreganophosphorus

pesticide extensively used an agricultural operation all over the world since 1956. Phosphamidon was first synthesized in 1955 by E. Benger of ClBA limited, Basle, Switzerland. Technical grade material is dark brown and commercial product is bright violet due to addition of a dye. It occurs in two isomeric forms a-and b-in the ratio of 3:7. Its insecticidal property is mostly due to b-isomer. It is a systemic poison and also acts as stomach poison. It has relatively low contact action. It is commonly used against insects possessing piercing and sucking, and chewing mouth-parts. It is widely used for the control of yellow borer and other sucking pests. There are mainly three routes of entry into water sources. One is from industrial waste or effluent discharged directly into water. A second is by seepage from buried toxic wastes into water supplies. Thirdly, contamination of running water directly or from run-off during spraying operations can occur. Mostly due to rainfall and drainage the pesticide residue reach the water bodies of many non target organism causing toxic effect on fishes.

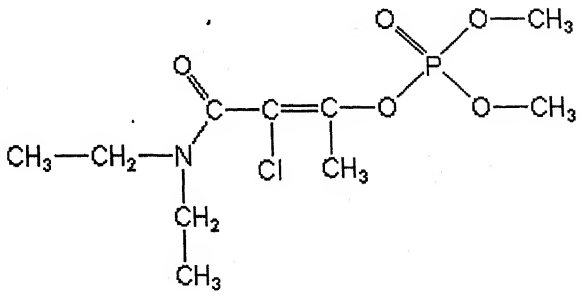
Primary Use: Insecticide

IUPAC : 2-chloro-2-diethylcarbamoyl-1-methylvinyl dimethyl phosphate

Common name : Phosphamidon

Molecular formula : $C_{10}H_{19}ClNO_5P$

Structural formula :



Trade names : Phosphamidon, Famfos, Dimecron etc.

Physical properties: -

Phosphamidon is a pale yellow to colorless oily liquid with a faint odour. It has a boiling point of 162°C at 1.5 mmHg. Phosphamidon exists as a mixture of 70% cis-isomer and 30% trans-isomer and is corrosive to iron, tinplate and aluminum and it is miscible with water. It is soluble in aromatic hydrocarbons but insoluble in non polar aliphatic hydrocarbons.

Mode of action: -

Phosphamidon poisoning inhibits the activity of superoxide dismutase and increases the lipid peroxidation in several regions in the central nervous system (Brain O' 1987). Delayed neuropathy is initiated by an attack on a nervous tissue system. The target has esterase activity and is called neuropathy target esterase (NTE). The disorder develops not because of loss of esterase activity, but because of a change brought about in the protein molecule that results from the process of ageing of inhibited NTE. The catalytic activity of NTE appears in the nervous tissue, even during the period of development of neuropathy (Edward et al., 1991). Investigations conducted by Extoxnet (1985); Matsumura et al., (1985); Rend G.M. and S.R., Petrocelli (1985) in fishes showed that phosphamidon is potentially neurotoxic because of its ability to inhibit brain NTE activity.

Oraganophosphorus pesticides exert their acute effects by inhibiting acetyl cholinesterase in the nervous system with subsequent accumulation of toxic levels of acetylcholine. They may also inhibit butyl cholinesterase as well as other esterase. The function of butyl

cholinesterase is unknown, but its inhibition can provide an indication of exposure to an organophosphate.

Metasystox

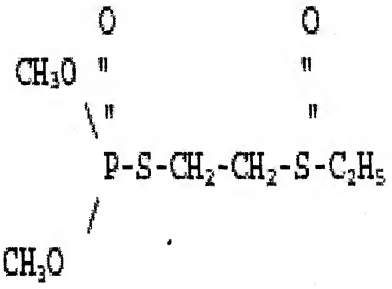
This pesticide was evaluated in 1965 by Joint Meeting of the FAO Committee on Pesticides in Agriculture and the WHO Expert Committee on Pesticide Residues (FAO/WHO, 1965) under the name of Oxydemeton-methyl. (ODM) Oxydemeton-methyl, *S*-[2-(ethylsulfinyl)ethyl], which is most commonly sold under the trade name of Metasystox R, is a systemic contact insecticide with approximately 120 tones applied in the states of India during 2004. Approximately 50% of the total was applied to broccoli, and 20% was applied to cauliflower during the year. It was assumed that ODM would be detected in air samples near application sites due to its relatively low vapor pressure (3.80 k Pa), but would not be found long distances from application sites. Dioxydemeton-methyl is a potential transformation product of ODM in air samples.

Primary Use : Insecticide, Oxydemeton-methyl

IUPACname : O,O-dimethyl-S-2-(ethyl-sulfinyl)-ethyl / phosph orothioate

Molecular formula : $C_6H_{15}O_4PS_2$

Structural formula :



Tread name : Metasystox

Physical properties

The molecular formula of metasystox is $C_6H_{15}O_4PS_2$ and its molecular weight is 246.3. it is clear amber-colored liquid (pure compound). The melting point is 10 C (pure compound), boiling point is 106 C/0.01 mm Hg (pure compound) and it is miscible in water.

Mode of action

Oxydemeton-methyl (Metasystox R) is an organophosphate pesticide. The target organ is nervous system and inhibiting cholinesterase enzyme. It binds to the enzyme that is normally responsible for breaking down Ach. When an insect has been poisoned by a cholinesterase inhibitor, the cholinesterase is not available to help breaking down the ACh, and the neurotransmitters continue to cause the neuron to "fire" or send its electric charge. This causes over stimulation of the nervous system, and the insect die.

Imidacloprid

Imidacloprid was discovered in 1984 at Nihon Bayer Agrochem in Japan by screening novel synthetic compounds for a high affinity to the insect nicotinic AChRs receptors, but with low toxicity to vertebrate species (Kagabu 1997). Its molecule includes the insecticidal N-(3pyridinyl) methyl group of nicotine and a nitroimine moiety, Because of their structural similarity to nicotine, imidacloprid and related insecticides (acetamiprid, thiacloprid, thiamethoxam and nitenpyram) were termed neonicotinoids (Tomizawa and Yamamoto, 1993). Imidacloprid was first registered in U.S. in 1994 as a pesticide

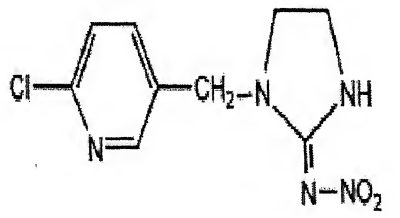
and developed for commercial use. Its major manufacturer is Bayer Corporation that markets imidacloprid products with the brand names Admire, Advantage, Confidor, Gaucho, Supreme etc.

Imidacloprid is a systemic chloronicotinyl insecticide that enters the target pest via ingestion or direct contact. In the environment, the principal routes of dissipation for imidacloprid are aqueous photolysis, microbial degradation and uptake by plants. The major degradation product of imidacloprid in the environment is desnitro-imidacloprid. Imidacloprid is used for controlling sucking insects, soil insects, termites, and some chewing insects.

Primary Use : Insecticide

IUPAC name : 1-(6-chloro-3-pyridylmethyl)-Nnitroimidazolidin-2-ylideneamine) is a synthetic active ingredient used in various insecticide

Structure formula



$C_9H_{10}C_lN_5O_2$

Physical properties:-

It is a colourless crystalline solid, has the molecular formula

$C_9H_{10}ClN_5O_2$ and a molecular weight of 255.7 (Tomlin 2000). Imidacloprid is soluble in water, relatively non-volatile.

Mode of action: -

Imidacloprid, and other insecticides in the nicotinoid chemical family, are "similar to and modeled after the natural nicotine [a tobacco toxin]. Because of their molecular shape, size, and charge, nicotine and nicotinoids fit into receptor molecules in the nervous system that normally receive the molecule acetylcholine. Acetylcholine carries nerve impulses from one nerve cell to another or from a nerve cell to the tissue. Imidacloprid and other nicotinoids irreversibly block acetylcholine receptors. It is used to treat seeds, soil, crops and structures, and is a flea control treatment on domestic pets (Meister, 2000). The toxicity of imidacloprid is based on interference of the neurotransmission in the nicotinic cholinergic nervous system. Imidacloprid binds to the nicotinic acetylcholine receptor (nAChR) at the neuronal and neuromuscular junctions in insects.

(b.) Physical properties of water: -

The chlorine free tap water was used through out the course of the experiment. The physiological characters of water sample like as the temperature of the test medium, pH, dissolve oxygen, alkinity, hardness and specific conductivity were tested in the Zonal laboratory U.P. Jal nigam Babina. Analyses of water have done during all three seasons.

Table : Physical properties of water

S. No.	Water Parameters	Rainy	Summer	Winter
1	Dissolve oxygen mg/liter	7.6	6.2	8.9
2	Alkinity as CaCo3 to methyl orange	326	320	308
3	Hardness as Ca	120	111.7	128.7
4	Specific condectivity micro mho	792	765	782
5	Water temprature °C	27	42	14
6	pH value	7.4	6.8	7.2

Chapter -5

EFFECT OF GLYPHOSATE ON BLOOD PARAMETERS OF *CHANNA PUNCTATUS*

EFFECT OF GLYPHOSATE ON BLOOD PARAMETERS OF *CHANNA PUNCTATUS*

Glyphosate 41% S.L. (trade name Round-up) was used in this study. The chemical composition of this compound is isopropyl amine salt of glyphosate. It is manufactured by Monsanto chemical of India limited, Mumbai.

Results

(a) Acute toxicity bioassay

In first exploratory test 100% mortality was observed in 0.030 ml/l of Glyphosate while no mortality occurred in 0.001 ml/l (Table.1). In second exploratory test four concentrations (0.007, 0.013, 0.019 and 0.025 ml/liter) were taken and mortality was recorded as shown in table 2. Eight different concentrations between 0.007 & 0.025 ml/l were left out for definitive test and mortality data were recorded (Table 3). Then a curve was plotted between percent mortality and concentrations of glyphosate used in definitive test. LC_{50} values were determined by drawing a line intersecting through the concentration of Glyphosate at 50% mortality level (Fig 1 and 2). The values of LC_{50} were estimated as:-

(1.) 24 hours-0.018 ml/liter

(2.) 48 hours-0.015 ml/liter

(3.) 72 hours-0.012 ml/liter

(4.) 96 hours-0.009 ml/liter

The behavioral changes were observed following exposure to

glyphosate. They show uncoordinated behaviour, agitated or erratic swimming excessive secretion of mucus. The colour of the tested fishes appeared little pale as compared to the normal fishes. Initially exposed fishes came to surface to engulf air frequently. After that the exposed fishes became very weak and settled at the bottom and died.

(b) Haematological & biochemical study:-

The haematological and biochemical parameters of control and glyphosate exposed fishes at LC_{50} concentrations are tabulated (Table 4). The Haemoglobin percentage and total erythrocytes counts were decreased significantly ($P < 0.001$). The Hb% in control fishes was 14.1 gm % where as in treated fishes it was 10.5, 10.7, 10.9 and 11.2 gm % at 24, 48, 72 and 96 hours respectively. The Hb % was lower at 24 hours LC_{50}. Although TEC was less from control fishes but gradually increased as exposure period increased but the difference between treated and control fishes at 96 hours LC_{50} was not significant. A significant decrease was also observed in PCV, MCH & MCV levels ($P < 0.01$). PCV was lowest at 24 hours and highest at 96 hours. MCH levels were also slightly increased from 24 – 96 hours exposure period. At 48 hours LC_{50} MCV was highest but at 96 hours it was lowest following exposure of glyphosate, but all these values were decreased when compared to control fishes. The TLC and MCHC levels increased significantly at $P < 0.01$. ESR was also increased from the control fishes. The ESR was 2.3 mm in control fishes and in herbicides treated fishes it was 3.3, 3.8, 3.8 and 3.6 at 24, 48, 72 and 96 hours LC_{50}, but the difference was insignificant at 48, 72 and 96 hours exposure period.

The table 4 shows that the level of glucose increased significantly ($P < 0.01$) in treated fishes. The level of glucose was 64.10 in control fishes where as fishes exposed to acute toxicity concentrations of Glyphosate showed 72.05, 74.19 74.10, 74.90 mg /100 ml blood of glucose concentration.

In chronic toxicity bioassay (Table 5) the Hb % TEC, PCV, MCH & MCHC decreased significantly ($P < 0.01$) after 15 days, 30 days and 45 days exposure of glyphosate (1/10 of 96 hours LC_{50} concentration). The Hb% was lowest after 15 days exposure period. The levels of other parameters such as TEC, PCV, MCH and MCV were also lowest after 15 days of exposure but gradually increased with increasing exposure period. A significant increase in the level of total leucocytes counts was observed ($P < 0.01$). Although an increased level of ESR was observed when compared with the unexposed fishes but statistically the difference was not significant. The table 5 indicates the changes in blood glucose level of *Channa punctatus*. Glucose level increased significantly ($P < 0.01$) when the fishes were subjected of glyphosate treatment. It was lower after 30 days exposure period.

(c) Study of seasonal variation:-

Seasonal changes of some haematological and biochemical parameters (glucose levels) are presented in the figures 3-11 during acute toxicity bioassay and in figures 12-20 in chronic toxicity bioassay. In acute and chronic toxicity tests the activity of haematological parameters (Hb%, TEC, TLC, ESR, PCV, MCV, MCHC and MCH) levels in blood were lower in late summer season while highest in rainy season. Although the blood parameters like

Hb%, TEC, PCV, MCV, MCH were decreased and TLC, ESR & MCHC were increased from the control fishes as shown in table 04 and 05 but the activity levels of these parameters were low in summer. The similar results have seen as in case of blood glucose level which increased in treated group when compared to untreated fishes but the level in treated and control group was lowest in summer season.

Discussion

(a) Acute toxicity:-

Formulations of Glyphosate including Rodco, Round up and Aquamaster have been extensively investigated (Monsanto Company 1985; Sawada Y., Nagai Y., 1987; Moses M., 1989; Tai et al., 1990; Tominack et al., 1991; Talbot et al., 1991). Glyphosate is used as non selective herbicides for aquatic weed control in ponds lakes and canals. The recommendation of field application rate usually ranges from 1500 and 2000 ppm in Asian countries. According to Wannee Jiraungkoorskul et al., (2002) the LC_{50} values of glyphosate (48 %) were 17.5, 17.1, 16.9 and 16.8 ppm at 24, 48, 72 and 96 hours after exposure on fresh water fishes *Oreochromis niloticus*. The acute toxicity of glyphosate (62 %) has been investigated by Mitchell et al., (1987) in rainbow trout using different dilutions and physiological conditions of water and the result shows that 96 hours. LC_{50} value ranges from 15-26 mg /liter. This result was also supported by Ayoola S.O., (2008). He calculated the LC_{50} values in *Oreochromis niloticus,* at 96 hours which was 1.05 mg/liter. Several works have also been carried out using glyphosate and other herbicides in fresh water fishes

(Brooker, M. P. and Edwards, R. W., 1975; Monsanto Company 1985; Sawada et al., 1987; Moses 1989; Anju Kumari and K., Pandey 1990; Tai et al., 1990).

Kumar Hemant and Gupta (1997) showed the effect of Glyphosate (Isopropyl amine salt of glyphosate) in *Heteropneustes* and estimated 146, 134, 129, & 124 ppm LC_{50} values at 24, 48, 72 and 96 hours. In the present study the LC_{50} values were 0.018, 0.015, 0.012 and 0.009 ml/liter at 24, 48, 72 & 96 hours which correlates the LC_{50} values of other workers. A review of literature revealed that, this is the first study made to evaluate the LC_{50} values of glyphosate for *Channa punctatus* in Bundelkhand region.

Several abnormal behaviour such as jumping and gulping of air, restlessness, sudden quick movement at the bottom in *Channa punctatus* were similar to the result of Omoniyi et al., (2002); Rehman et al., (2002); and Aguigwo J.N., (2002). The erratic and stressful behaviour of fishes in the present study indicated respiratory impairment, which may be due to the effect of glyphosate herbicides on the gills. (Vivekanandan E., Pandian T.J., 1977; Black et al., 1980; Baldwin et al., 1994; Allin, C.J., and R.W., Wilson. 2000; Weis, J.S., et al.,2001). Light microscopic study of the gills of *Nile tilapia* exposed to glyphosate showed several pathological changes and their frequencies increased with increasing the exposure time (Ayoola S.A., 2008). The fishes came to surface to engulf air. This may be due to low rate of Oxygen uptake under toxicity stress (Wilson, R.W., Taylor, E.W., 1993; Stein, J. E., et al., 1992; Vander et al., 2003).

(b) Haematological and biochemical study:-

Synthetic herbicides are commonly used by farmers to control weeds and nuisance aquatic vegetations around rivers, lakes and reservoirs. However, these herbicides find their way to water bodies, inducing adverse impact on fishes living their in (Tsuda et al., 1997). Since blood forms a unique component between the external and internal environment, toxicant that cause stress in fishes can alter the composition of blood. So that the use of haematological parameters for toxicological research is important for environmental monitoring and fish health conditions. Many works have been conducted on haematological changes of herbicides in fishes (USDA., 1984; Mitchell D.G., et al., 1987; Servizi J.A., et al., 1987; Singh H.S., Reddy T.V., 1990; Neskovic N., et al., 1993; Annune P.A., et al., 1994; Risbourg, S.B., 1995; Rahman M.Z., 2002; Ayoola S.O., 2008). The significance reduction in the values of haemoglobin percentage and TEC after exposure to lethal and sub lethal concentration is an indication of anaemia caused by glyphosate on the exposed fishes. The inhibition in the level of Hb% and TEC were also observed by Kori Siakpers., (2007) using the herbicides paraquet. Significant decrease occurred in the Hb% level may be due to the impairment of oxygen supply to various tissues especially gills. (Ayoola S. 2008; Neskoric N.K., et al., 1996; Rojik J., et al., 1983). Reduced TEC may be as a result of anaemia which is due to haemodilution resulting from impaired osmoregulation across the gill epithelium as reported by Svododa et al., (2001).

Increased in TLC has been attributed to several factors such as increase in thrombocytes and lymphocytes (Agrawal and Shrivastava 1980; Thakur G.K. and P.K., Pandey 1990). Leucocytes are involved

in the regulation of immunological functions of the body (Santa Kumar et al., 2000). An increased in TLCs thus occurs as a protective response to stress (Das B.K., 1998). In the present study PCV value decreased when the fishes are poisoned by toxicant (Gill and Pant 1985). The reduction indicates that the fishes suffer from anaemia or haemodilution (Wedemeyer et al., 1976). Fall in MCV, MCH and increase MCHC values were observed in the present study in acute and chronic conditions. This alteration confirms anaemia in fishes. These alterations also may be due to histopathological changes occur in RBC following exposure to toxicants in *Channa punctatus* and other fresh water fishes (Dalela R.C., et al., 1979; Sastry K.V., and K., Sharma 1981; Dange A.D., 1986; Gill T.S., Pant,J.C., and Pant J., 1988; Hinton D.E., & Lauren D.J., 1990; Birendra Kumar and Banarjee V., 1991; Nowak B., 1992; Poleksic V., et al., 1994; Brusle J., et al., 1996; Ecobichon et al., 1996; Kashiwada, S., et al., 2002; Choudhary et al., 2003; Vander Oost R., et al., 2003; Capkin E., et al., 2006). Increased level in values of ESR may be due to increase concentration of fibrinogen which develops fibrinogenemia due to various pesticides exposure (Singh S. and Bhati DPS 1991; Mala et al., 2009).

Biochemical characteristics are among the important indices of the status of internal environment of the fishes (Nemcsok and Bones 1982; Edsall C.C., 1999). Changes in the biochemical parameters cause changes in metabolism due to the effect of various pollutants. Changes in the activity of several enzymes and carbohydrate metabolism have been observed by several workers (Hochachka, P.W., 1978; Ransfold K. D. 1978; Jagdish et al., 1981; Shrivastva A.

K., and Singh N. N. 1981; Shobha et al., 1989; Chandrasekhar and Jayabalan S. 1993; Jyothi B., and Natrajan G. M. 1999; Prakash et al., 2007). A significant increase in blood glucose level or hyperglycemia occurs in glyphosate treated fishes. The blood glucose level in Indian cat fishes *Heteropneustes fossilis* exposed to sub lethal concentrations of other toxicants were also found to increase considerably (Love R. M., 1970; Dragomieaseu et al., 1975; Ransfold K. D., 1978; Jagdish et al., 1981; Shrivastva A. K., and Singh N. N. 1981; Shobha et al., 1989; Chandrasekhar and Jayabalan S. 1993; Hemant and Gupta A.B. 1997; Kumar; Prakash et al., 2007).

Increased in blood glucose level may indicate a greater energy requirement of the fishes due to glyphosate stress. The stress may also be viewed as a response of respiratory insufficiency (Rahman M.Z., 2002; Ayoola S.O., 2008). Altered carbohydrate metabolism and impairment in the liver of fishes may be another reason for hyperglycemic condition found in *Channa punctatus* following exposure to glyphosate.

(c) Seasonal variation:-

The data presented in figure 3-11 and 12-20 showed that haemoglobin percentage, TEC, PCV, MCH and MCV decreased but TLC, ESR, MCHC and blood glucose level were increased following exposure to glyphosate from control fishes both in acute and chronic experiment. Although these changes were similar as shown in table 4 and 5, but the concentrations of above parameters in control and treated fishes were lowest in summer and then increase in rainy season. This clearly shows that a process of haemoconcentration

occurs through the late spring and summer and that is followed by haemodilution in the late summer. ESR which is the measure of the concentration of the blood rate, shows down in summer and increases as the blood becomes dilute. A similar response of haemoconcentration and haemodilution is described in fishes and other cold blooded animals like frogs (Schaefer A.A., 1925; Kaplan and Carousa 1956). The lower values in summer may also be due to low rate of oxygen during summer season. (Harding J., and Hogland, L. B.,1984; Joshi P.C.,1989; Akira Kakuno and Jiro Koyama 1994; Dento, J.E., and Yousef M.K., 1995; Gross et al., 1996; Collazos M.E., et al., 1998; Best et al, 2001). The chemical investigations of water in the present study (chapter 4) shows that the low rate or dissolve oxygen in summer season.

Determination of LC_{50} for Glyphosate on *Channa punctatus*

Table 01: First exploratory test

S.No.	Conc. ml/liter	No. of Fishes	24 Hours		48 Hours		72 Hours		96 Hours	
			M	%M	M	%M	M	%M	M	%M
1.	0.001	5	0	0	0	0	0	0	0	0
2.	0.030	5	5	100	-	-	-	-	-	-

Table 02: Second exploratory test

S.No.	Conc. ml/liter	No. of Fishes	24 Hours		48 Hours		72 Hours		96 Hours	
			M	%M	M	%M	M	%M	M	%M
1.	0.007	5	0	0	0	0	1	20	1	40
2.	0.013	5	1	20	1	40	1	60	1	80
3.	0.019	5	3	60	1	80	1	100	-	-
4.	0.025	5	5	100	-	-	-	-	-	-

Table 03: Definitive test

S.No.	Conc. ml/liter	No. of fishes	24 Hours		48 Hours		72 Hours		96 Hours	
			M	%M	M	%M	M	%M	M	%M
1.	0.008	10	0	0	1	10	1	20	2	40
2.	0.010	10	0	0	2	20	2	40	2	60
3.	0.012	10	1	10	2	30	2	50	2	70
4.	0.014	10	2	20	2	40	2	60	3	90
5.	0.016	10	3	30	3	60	3	90	1	100
6.	0.018	10	5	50	3	80	1	90	1	100
7.	0.020	10	7	70	2	90	1	100	-	-
8.	0.022	10	8	80	2	100	-	-	-	-

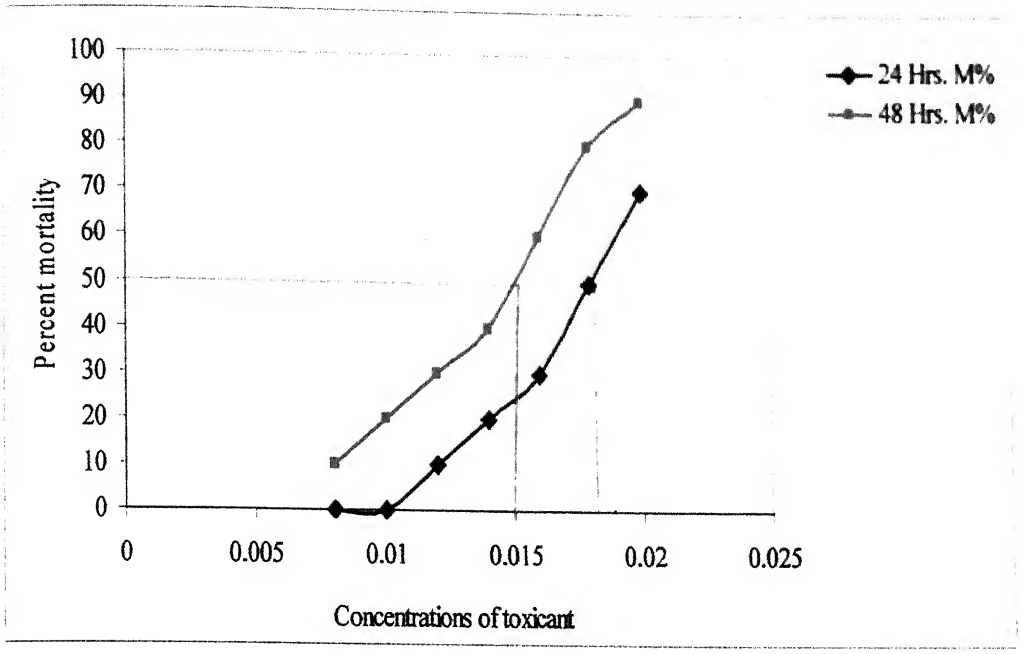


Fig 01: LC_{50} of Glyphosate after 24 hours and 48 hours

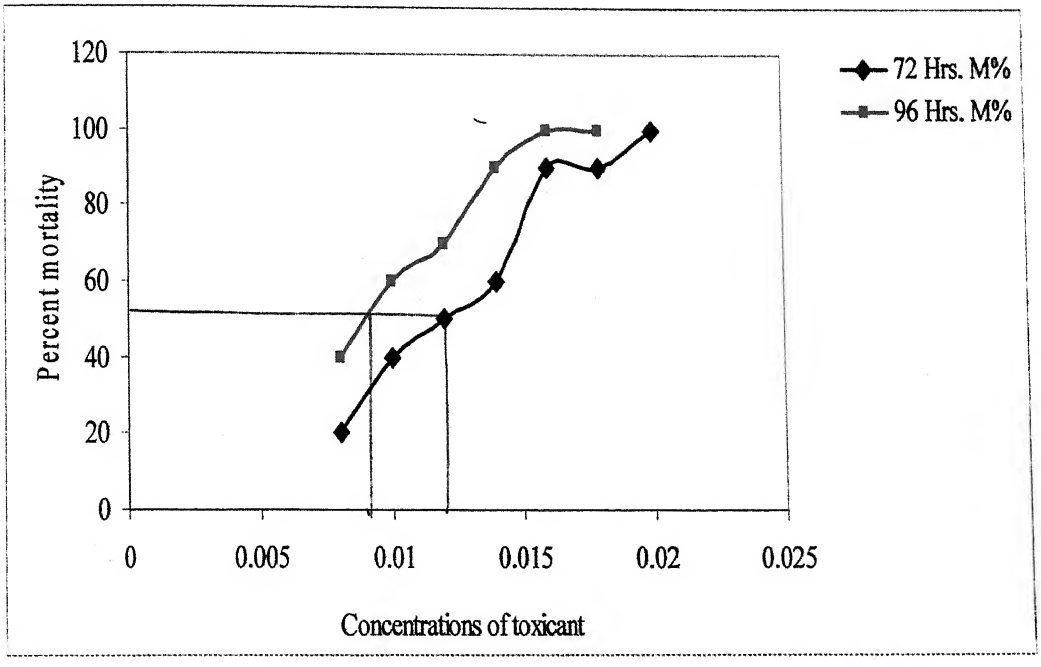


Fig 02: LC_{50} of Glyphosate after 72 hours and 96 hours

Table No. 04

Effect of acute toxicity of Glyphosate on selected blood parameters in fresh water fish *Channa punctatus*

S No.	Parameters	Control	Exposure Period			
			24 Hours	48 Hours	72 Hours	96 Hours
01.	Hb (g/100ml)	14.1 ±0.72	10.7** ±0.52	10.7** ±0.43	10.9** ±0.70	11.2** ±0.25
02.	TECx10^6/mm^3	3.84 ±0.06	3.46** ±0.05	3.47** ±0.07	3.48** ±0.09	3.45 ±0.02
03.	TLCx10^3/mm^3	3.2 ±0.51	3.7* ±0.72	4.2* ±0.3	4.0* ±0.46	3.9* ±0.64
04.	ESR (mm)	2.3 ±0.76	3.3* ±0..28	3.8** ±0.28	3.8** ±0.28	3.6** ±0.28
05.	PCV%	33.0 ±3.0	22.66* ±1.15	24.33* ±1.52	24.66* ±2.88	26.00* ±2.64
06.	MCH pg	36.78 ±2.21	30.85* ±1.21	30.80* ±1.27	31.35* ±1.90	33.26* ±0.67
07.	MCHC %	42.59 ±1.79	47.20* ±0.46	44.13* ±4.21	44.68* ±5.58	43.63* ±4.70
08.	MCV um^3	85.87 ±7.86	70.09* ±10.65	73.80* ±9.73	66.92* ±0.48	65.60* ±9.63
09.	Glucose (Units)	64.10 ±6.82	72.05* ±7.58	74.19* ±8.63	74.10* ±8.49	74.90* ±8.36

* - Significant at $P < 0.01$; ** - Significant at $P < 0.001$

Table No. 05

Effect of chronic toxicity of Glyphosate on selected blood parameters in fresh water fish *Channa punctatus*

S No.	Parameters	15 Days		30 Days		45 Days	
		C	T	C	T	C	T
01.	Hb (g/100ml)	13.9 ±0.60	12.5* ±0.55	14.0 ±0.51	13.4* ±0.50	14.4 ±0.60	13.3* ±0.51
02.	TECx10^6/mm^3	3.77 ±0.11	3.58* ±0.91	3.90 ±0.04	3.63* ±0.09	3.98 ±0.09	3.88* ±0.09
03.	TLCx10^3/mm^3	3.1 ±0.40	3.8* ±0.37	3.2 ±0.35	4.0* ±0.35	3.5 ±0.40	4.4* ±0.10
04.	ESR (mm)	2.3 ±0.28	3.9** ±0.11	2.5 ±0.11	4.1** ±0.41	2.2 ±0.32	4.2** ±0.23
05.	PCV%	27.33 ±2.30	19.33* ±3.05	30.00 ±3.60	21.66* ±2.51	32.33 ±2.30	24.33* ±2.08
06.	MCH pg	36.85 ±0.47	34.71* ±0.70	36.10 ±1.52	35.43* ±0.67	36.84 ±0.60	36.30* ±0.36
07.	MCHC %	51.08 ±4.71	65.14* ±8.12	47.46 ±6.20	59.73* ±4.55	44.35 ±3.30	56.24* ±3.05
08.	MCV um^3	72.53 ±6.27	53.87* ±7.21	76.93 ±10.17	59.59* ±5.45	83.36 ±6.42	65.87* ±4.05
09.	Glucose (Units)	60.54 ±7.22	72.36* ±10.32	63.34 ±6.71	68.98* ±5.04	65.14 ±5.25	76.75* ±6.47

* - Significant at $P < 0.01$; ** - Significant at $P < 0.001$

Graphical comparison of seasonal variation in acute toxicity experiment

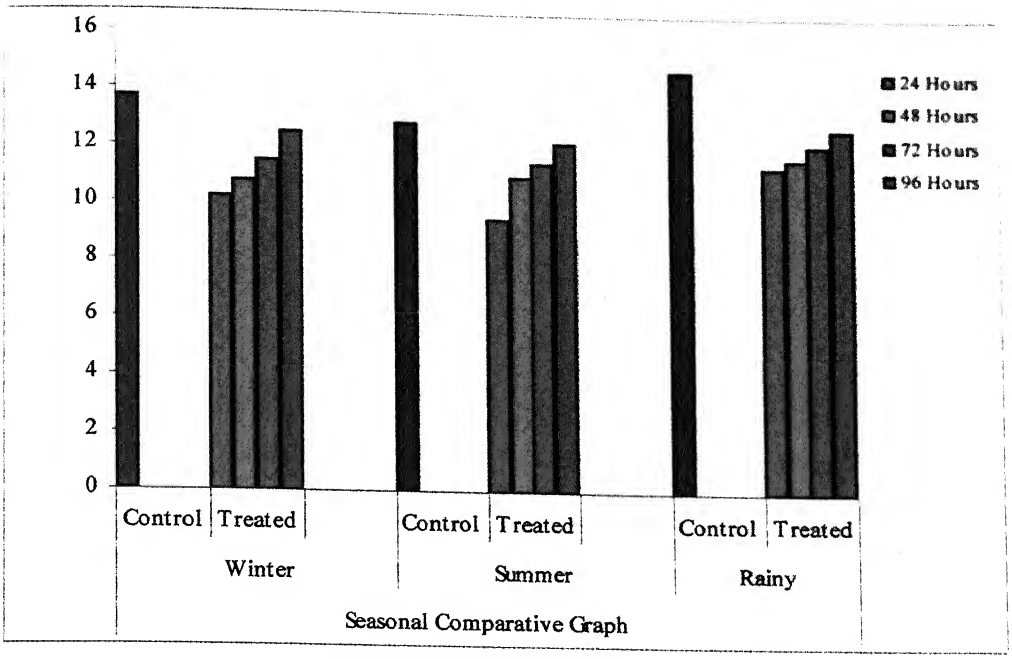


Figure 03. Haemoglobin

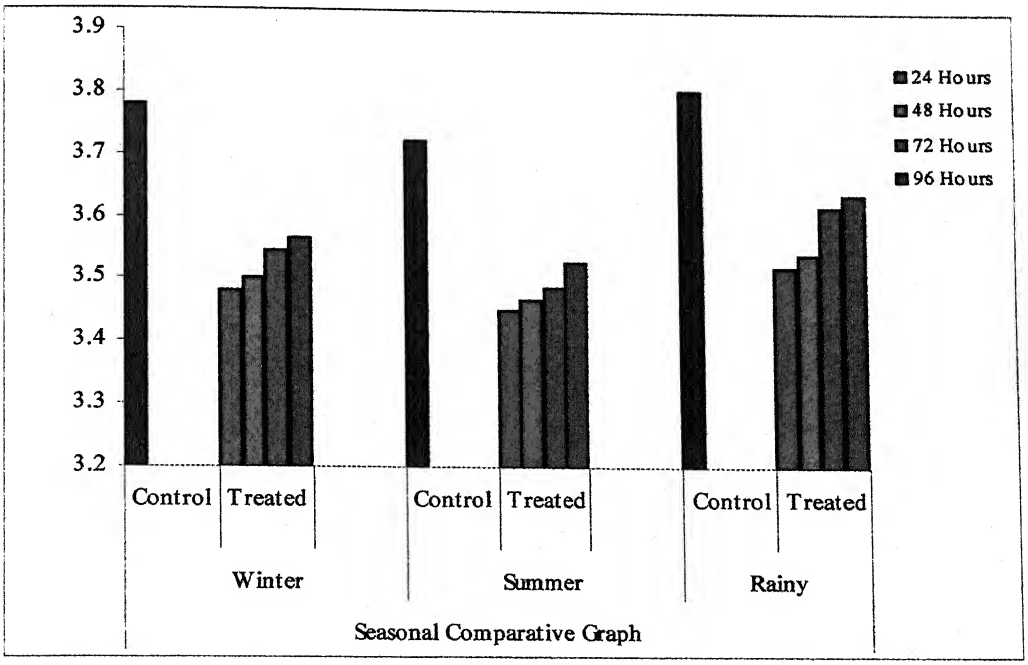


Figure 04. TEC

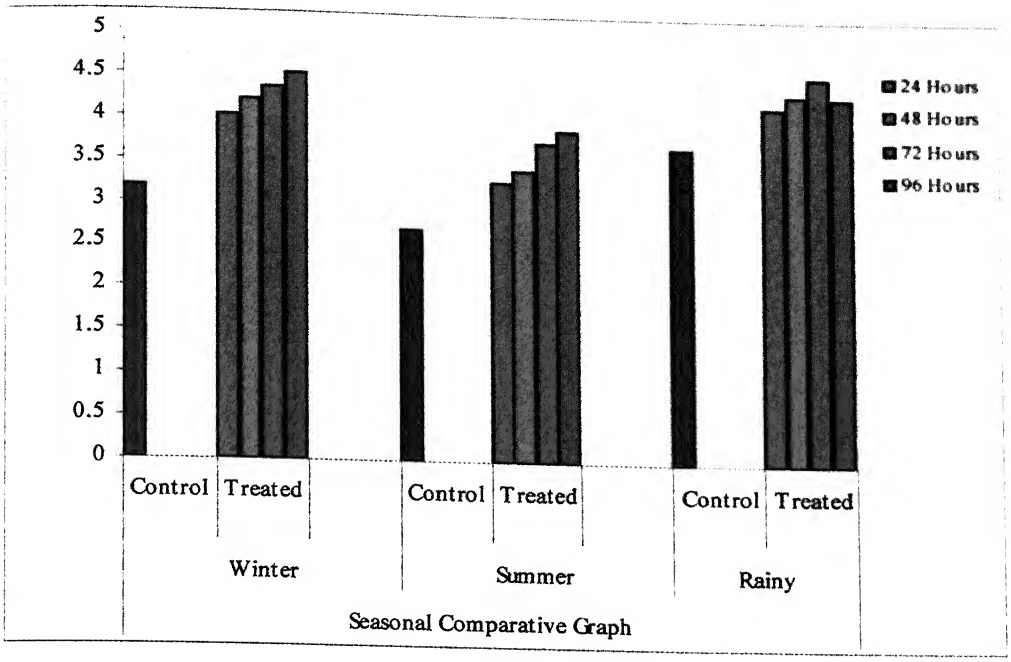


Figure 05. TLC

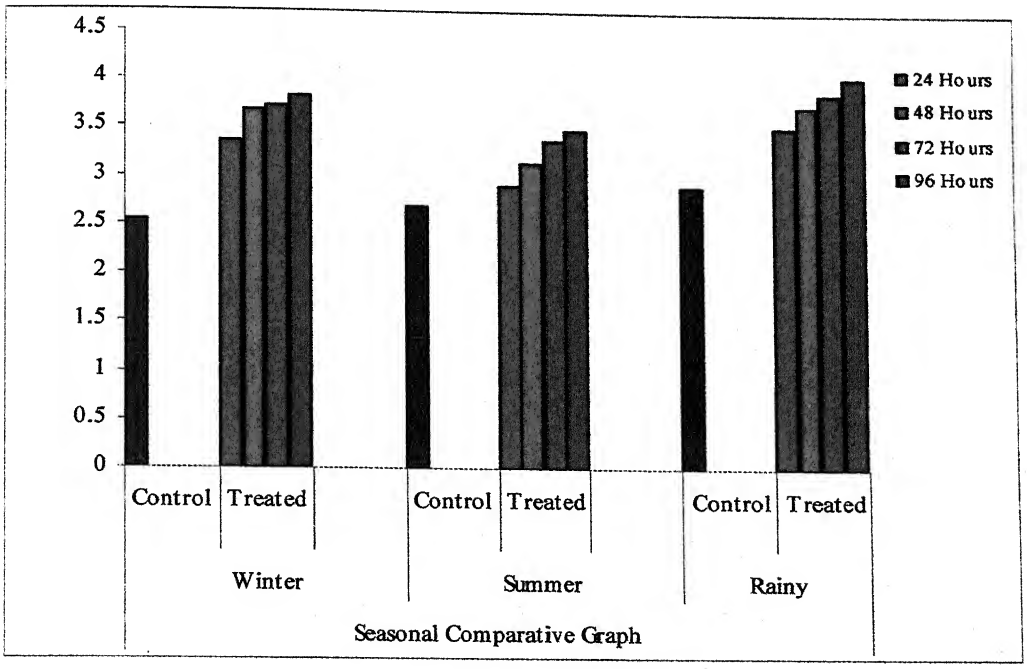


Figure 06. ESR

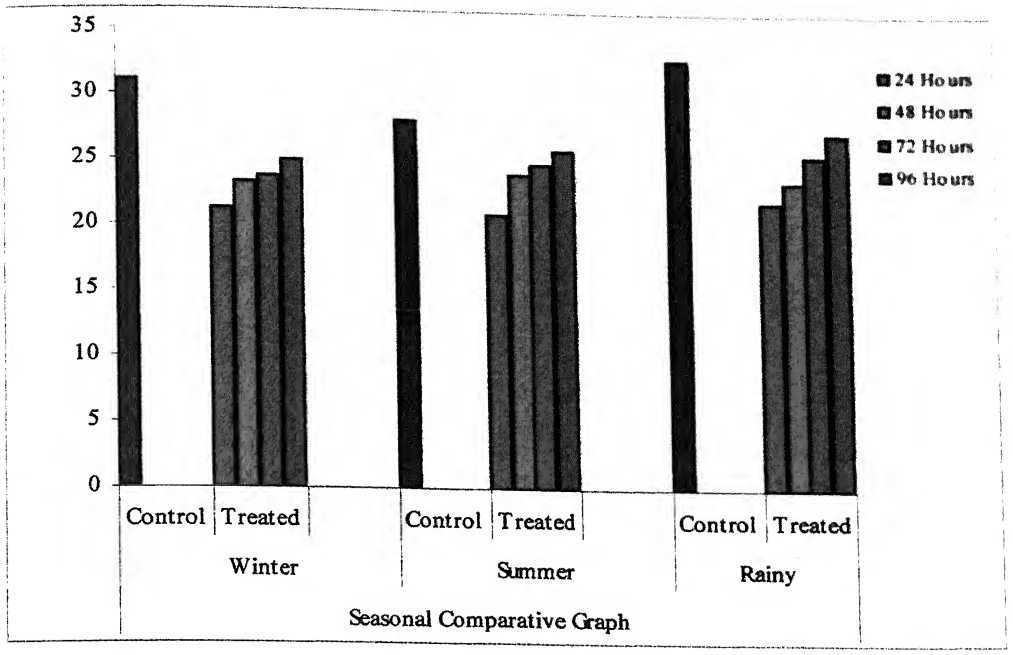


Figure 07. PCV

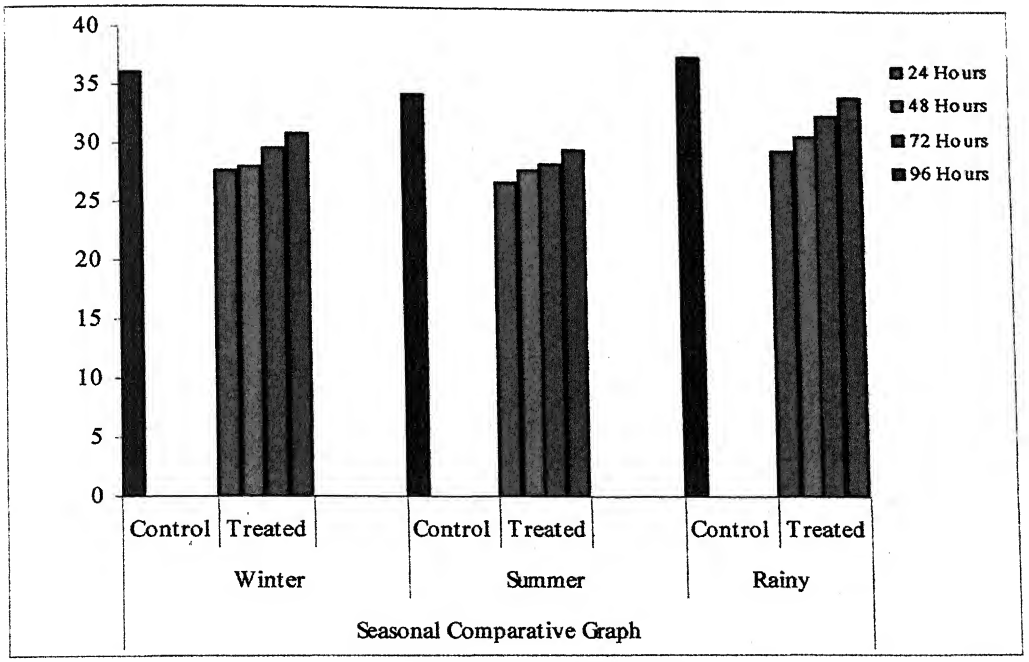


Figure 08. MCH

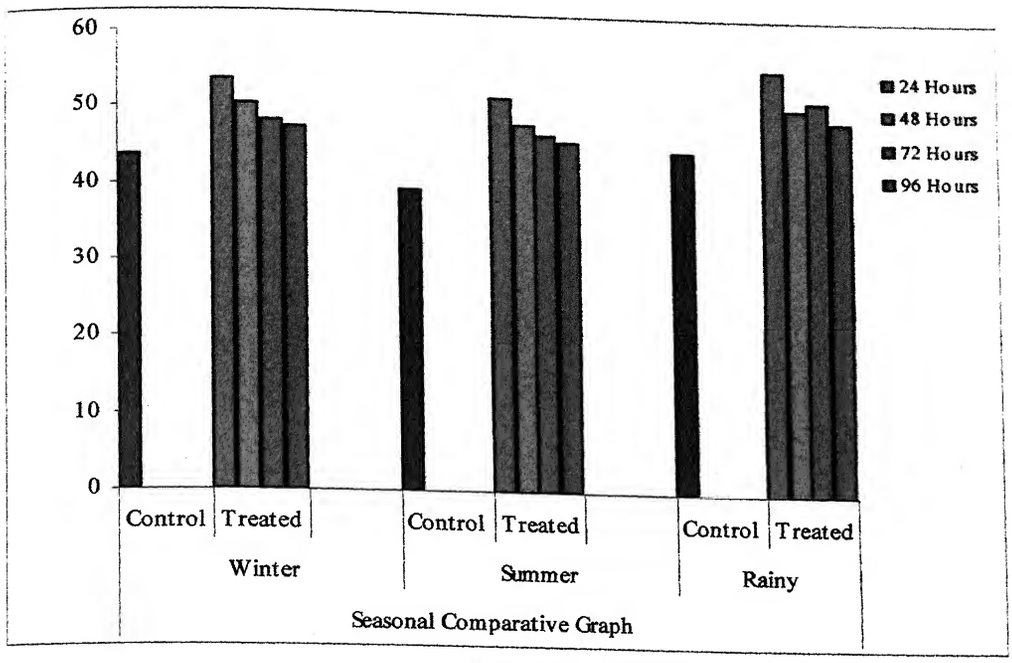


Figure 09. MCHC

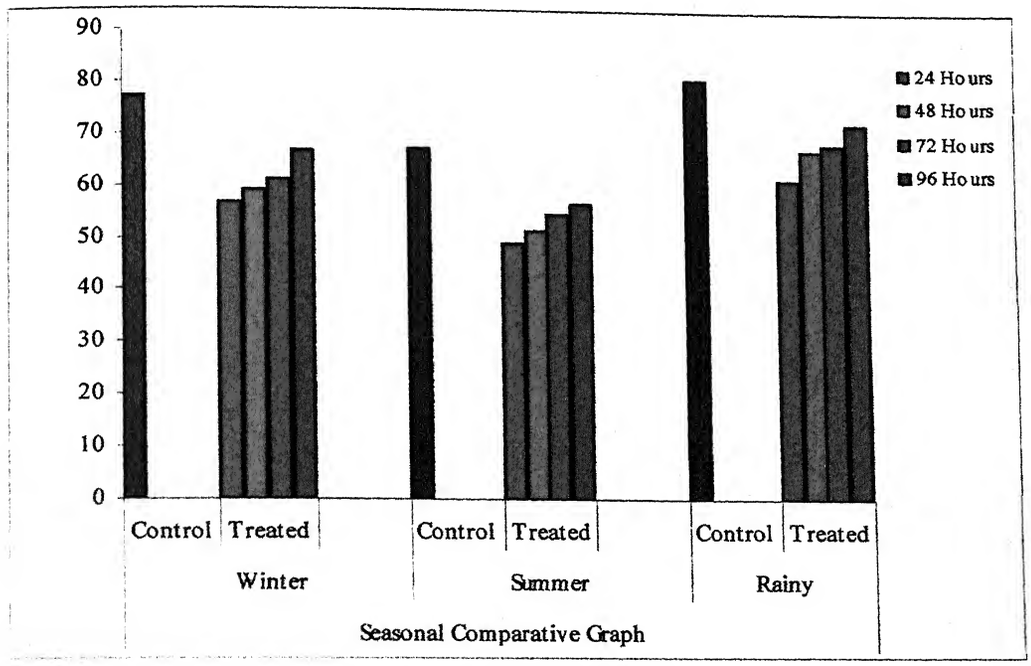


Figure 10. MCV

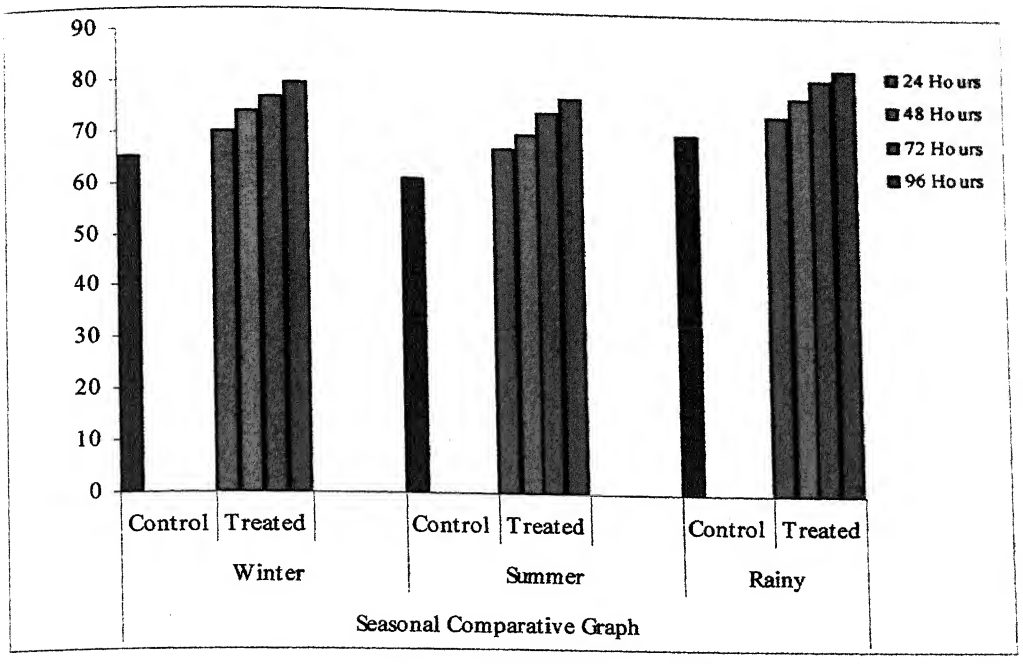


Figure 11. Blood Glucose

Graphical comparison of seasonal variation in chronic toxicity experiment

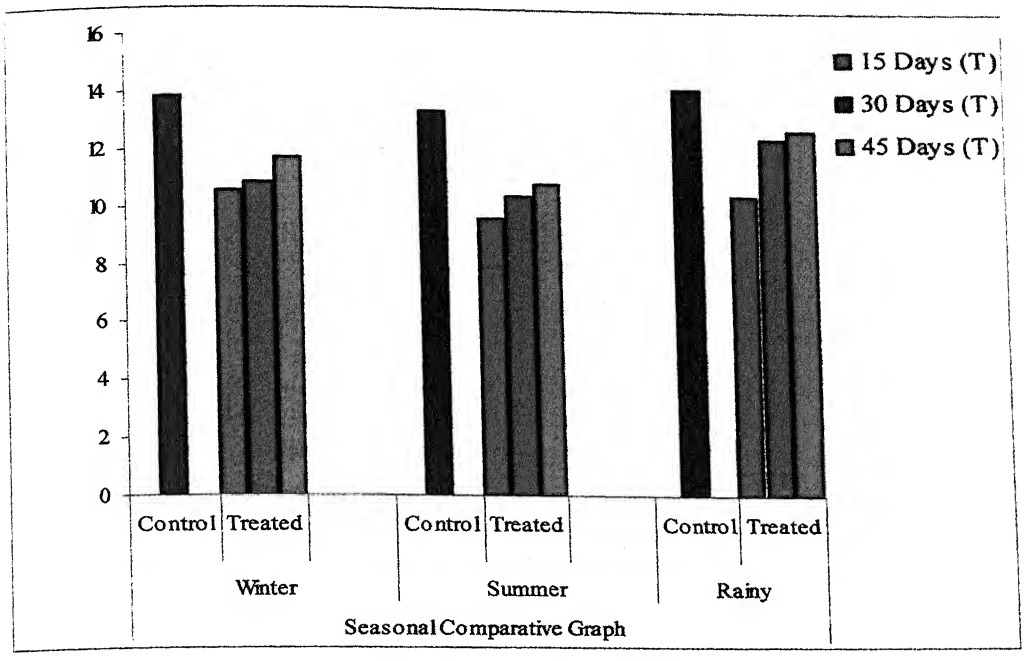


Figure 12. Haemoglobin

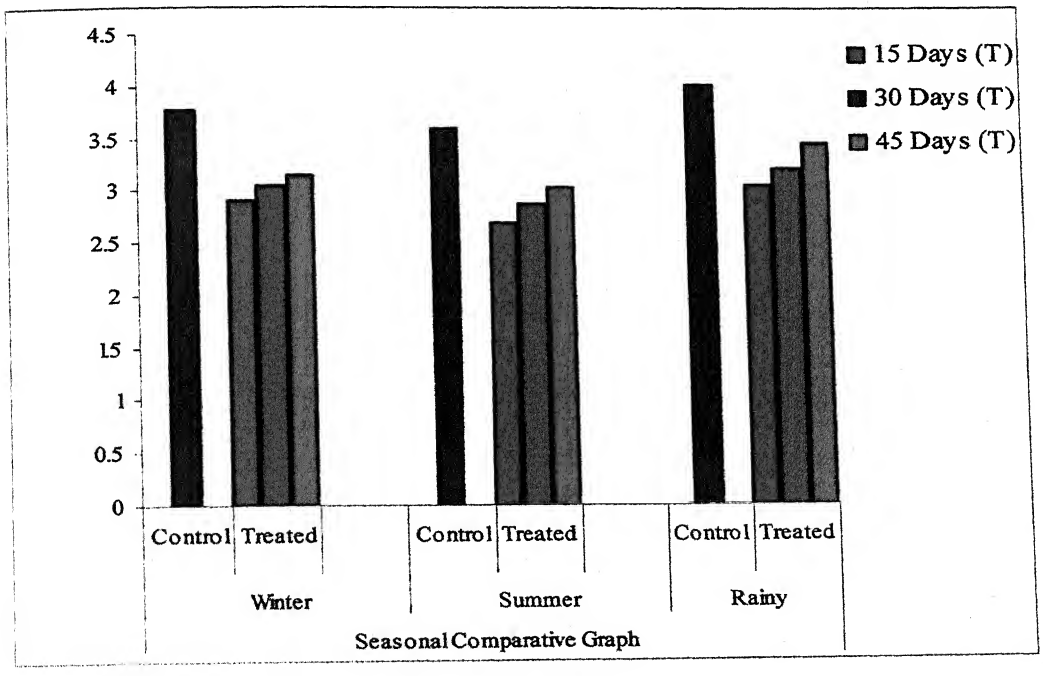


Figure 13. TEC

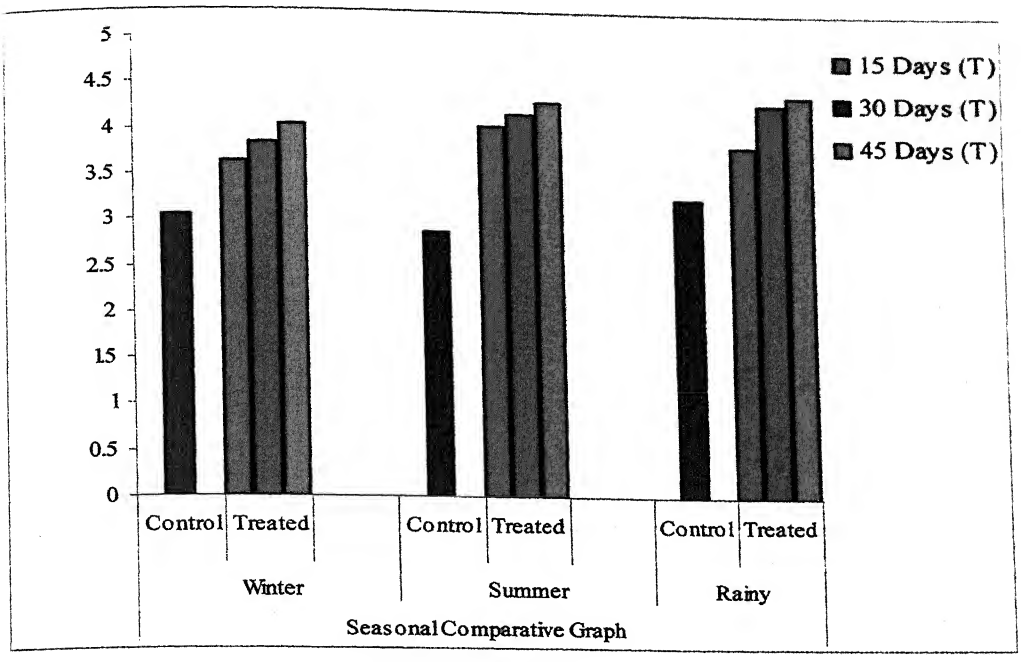


Figure 14. TLC

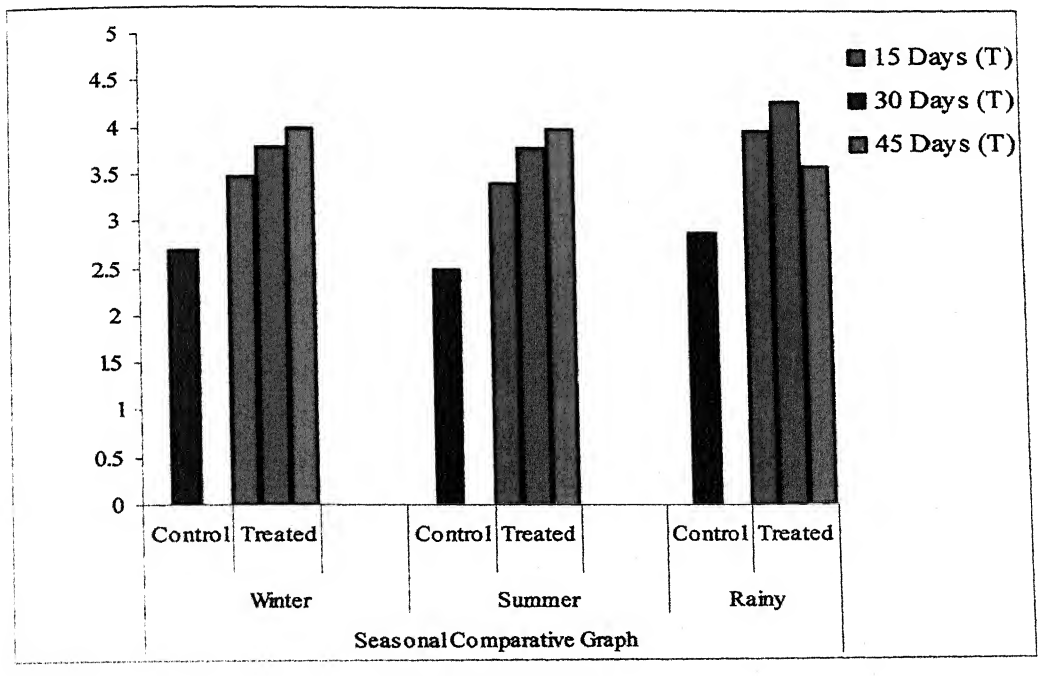


Figure 15. ESR

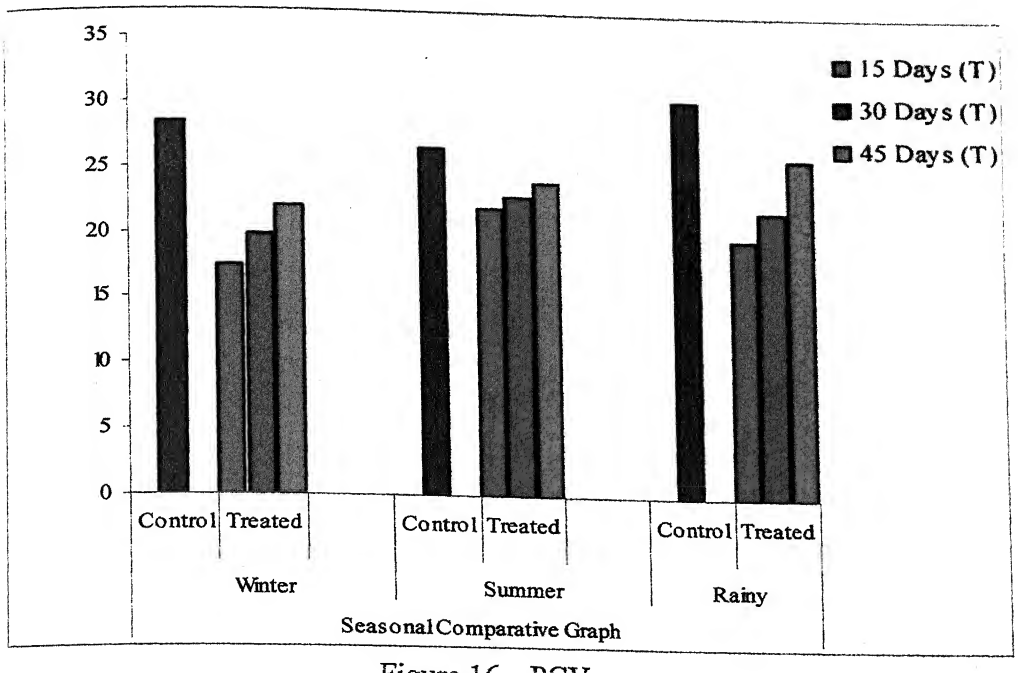


Figure 16. PCV

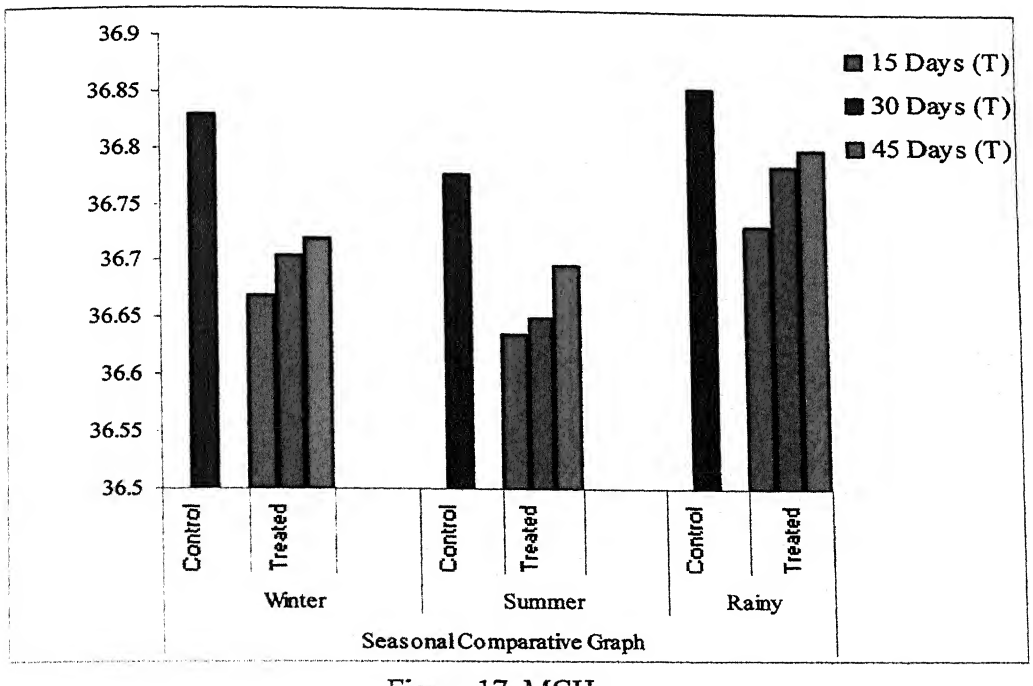


Figure 17. MCH

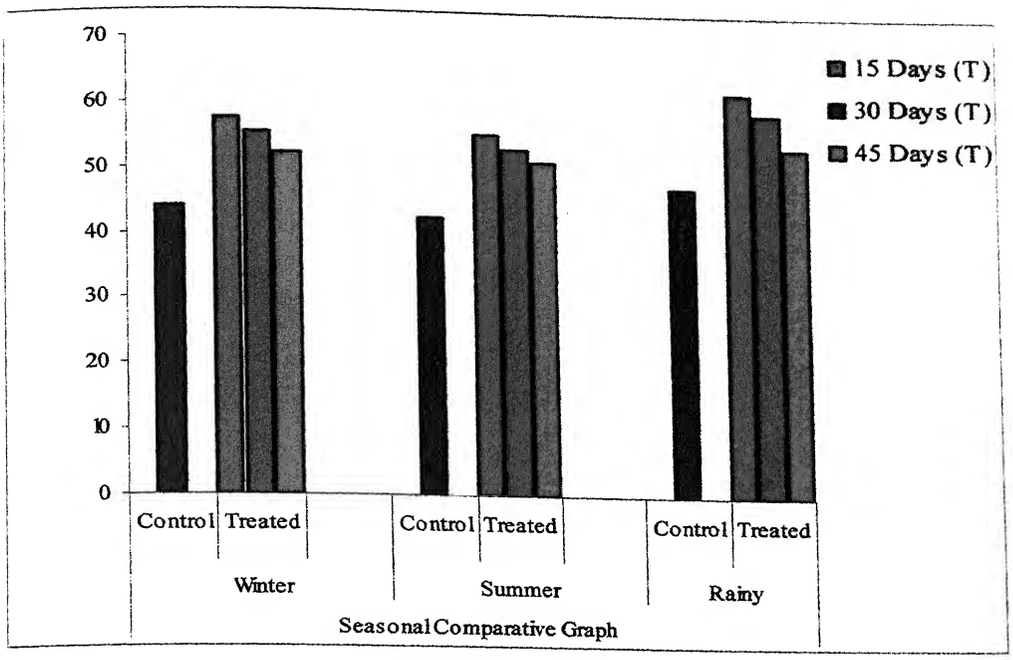


Figure 18. MCHC

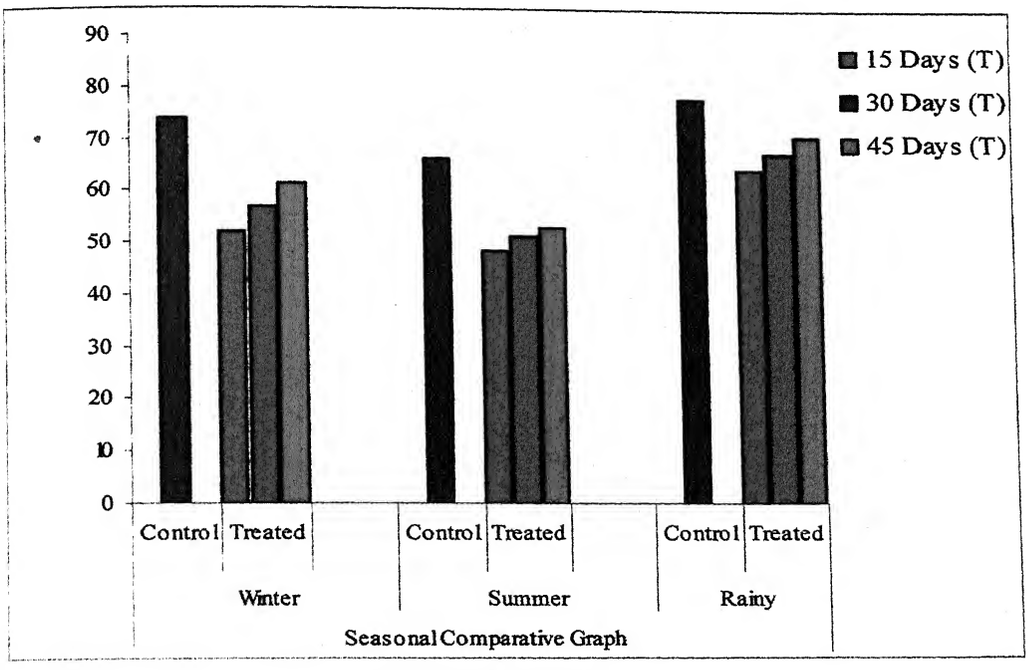


Figure 19. MCV

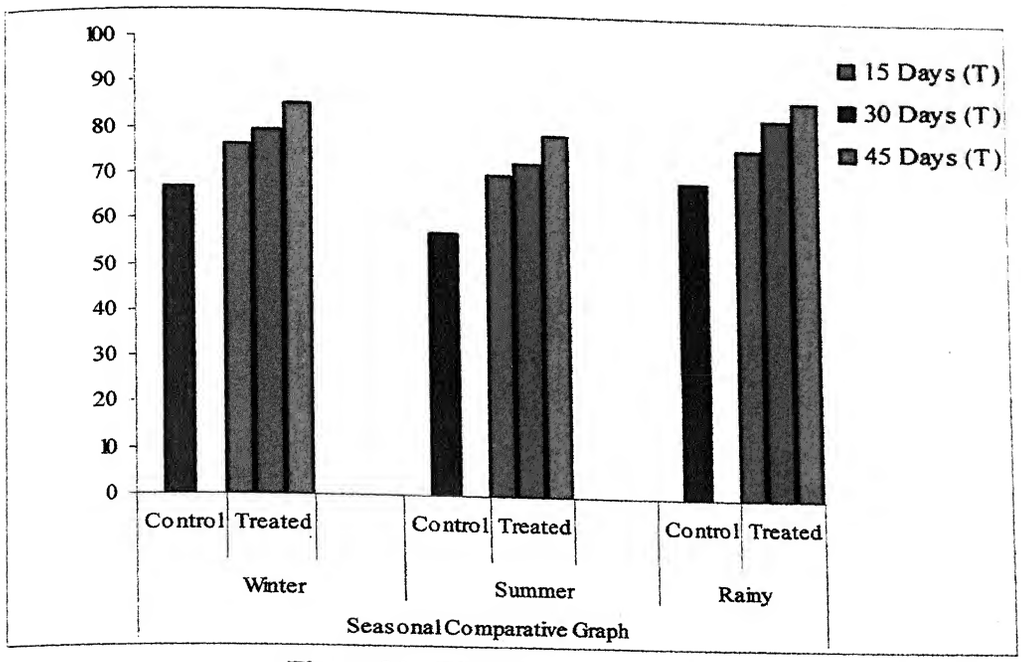


Figure 20. Blood glucose

Chapter - 6

EFFECT OF PHOSPHAMIDON ON BLOOD PARAMETERS OF *CHANNA PUNCTATUS*

EFFECT OF PHOSPHAMIDON ON BLOOD PARAMETERS OF *CHANNA PUNCTATUS*

Phosphamidon 41% S.L. (trade name Don 400) insecticide was used in this investigation. The chemical composition of this compound is O-(2-chloro-2-diethylcarbamoyl-1-methyl (vinyl) dimethyl phosphate. It is manufactured by Devi dayal (sale) limited, Reay road, Mumbai.

Results

(a.) Acute toxicity bioassay:-

In first exploratory test 100% mortality was observed in 0.04 ml/liter and no mortality in 0.002 ml/l (Table 06). In second exploratory test mortality due to different concentrations were observed (Table 07). The concentrations were (0.007, 0.014, 0.021 and 0.028 ml/liter). The concentrations between 0.007 ml/l & 0.028 ml/l were left out for definitive test because 100% mortality was observed in 0.028 ml/liter at 48 hours and 20% mortality was seen in 0.007ml/liter at 96 hours. Nine concentrations (0.008, 0.010, 0.012, 0.014, 0.016, 0.018, 0.020, 0.022, 0.024 ml/liter) were selected for definitive test and mortality data were recorded in table 08. Then a curve was plotted between percent mortality and concentrations of phosphamidon using definitive test, LC_{50} values were determined by sketching a line intersecting through the concentration of phosphamidon at 50% mortality altitude. (Fig 21 and 22). The values of LC_{50} were estimated as

(1.) 24 hours-0.023 ml/liter

(2.) 48 hours-0.019 ml/liter

(3.) 72 hours-0.015 ml/liter

(4.) 96 hours-0.011 ml/liter

The behavioural changes were observed following exposure to phosphamidon. It was restlessness, erratic and jerky movement, and loss of balance, jumping and breathing rapidly. Other changes like as the extra secretion and deposition of mucus over the surface of body and fading of body colour has also been observed. The exposed fishes became very weak, settled at the bottom and ultimately died.

(b) Haematological and biochemical study:-

The haematological and biochemical parameters of control (untreated) and phosphamidon exposed fishes at LC_{50} concentrations are tabulated (Table 09). The table 9 shows that the haemoglobin percentage (Hb %) and total erythrocytes counts (TEC) were decreased significantly ($P < 0.001$). Although the values of Hb% and TEC were all the times lesser than untreated fishes but at 72 hours the difference was statistically insignificant. The maximum value of Hb % was found in 24 hours and minimum in 96 hours when compared to control. The table 09 also confirms that the rate of TLC was always greater than untreated fishes, but progressively increased during 24 to 96 hours. MCHC % of blood significantly increased ($P < 0.01$) in fishes after acute exposure to insecticides compared to the corresponding control but it was observed that level of MCHC decreased progressively. The values of ESR were insignificantly increased, but at 96 hours it was significant ($P < 0.01$). The data illustrate that the value of PCV, MCH and MCV were significantly

decreased ($P < 0.01$), but it was also become apparent that the values of PCV, and MCV were lowest in 24 hours exposure and highest in 96 hours intoxication of phosphamidon.

The effect of biochemical parameter (B.glucose) after acute exposure of phosphamidon has been shown in table 09. It was clear that the values of glucose were increased significantly ($P < 0.01$) during treatment of fishes with phosphamidon. The level of blood glucose was 64.7 units in control fishes while 70.07, 73.71, 73.72 and 74.91 units for 24, 48, 72 and 96 hours respectively in treated fishes.

In chronic toxicity bioassay (Table 10) the Hb % TEC, PCV, MCH & MCV were decreased significantly ($P < 0.01$) at 15 days, 30 days and 45 days after exposure at 1/10 of 96 hours of phosphamidon LC_{50} concentration. It was observed that the levels of Hb%, TEC, MCH, PCV and MCV were less than control fishes, but gradually increased with rising exposure periods. The table 10 demonstrates that the levels of TLC, ESR and MCHC were increased significantly at $P < 0.001$.

The level of blood glucose after chronic exposure of phosphamidon has given in table 10. The level of blood glucose was increased significantly at $P < 0.01$. It was lower after 15 days but higher after 45 days exposure period.

(c) Study of seasonal variations:-

The seasonal alterations of selected haematological and biochemical parameters (glucose levels) were obtained and arrange in figure 23-31 during acute toxicity and figure 32-40 during chronic

toxicity bioassay. If comparison was made in control fishes during different seasons, it was found that in acute toxicity cases the activity of haematological parameters (Hb%, TEC, TLC, ESR, PCV, MCV, MCHC, MCH and blood glucose) level in blood were lower in summer season and higher in rainy season. Although the blood parameters like Hb%, TEC, PCV, MCV, MCH were decreased and TLC, ESR, MCHC and blood glucose were increased from the control fishes as shown in table no. 09 & 10, but the activity levels were moderate in winter, slightly high in rainy season and low in summer. Approximately comparable responses were originated in chronic toxicity test.

Discussion

(a) Acute toxicity bioassay:-

Organophosphorus insecticides (phosphamidon) are now extensively used in plant protection operation an accounts of their less persistence in the environment. Various bioresearchers have reported the LC_{50} value in different fishes intoxication of phosphamidon depending on their test conditions. Jaya Natha rao et al., (1984) notified the LC_{50} value (16.0 mg/liter) of phosphamidon at 48 hours in *Sarotherodon massambicus*. Sreenivasulu et al., (1991) calculated the13.0 ppm LC_{50} values of phosphamidon at 96 hours in *Tilapia mossambica*. Sexsena et al., (1997) reported the LC_{50} values 5.54 ppm for 96 hours in *Channa orientalis*, while Jeba kumar (1997) estimated the LC_{50} value 8.0 ppm for 96 hours in *Heteropneustes fossilis*. According to Imbamani and Shrivassan (1998) the LC_{50} value was 2.378 ppm for 96 hours in *S. mossambica*. However Anand Kumar A.,

and Tripathy N.K., (2001) reported 550 ppm and 280 ppm LC_{50} values for 24 and 96 hours in *Heteropneustes fossilis*. Some other workers have described toxicity of phosphamidon and other insecticides on fresh water fishes. (Mahesh et al., 1989; Sikoki F. D., & Enajekpo H.O.S., 1991; Ghosh R., Shrotri R .V., 1992; Stein et al., 1992; Singh et al., 1992; Canli M., 1996; Luskova V., 1997; Dhembare A.J., and Pondha G.M., 2000; Das B. K. and Mukherjee S. C., 2000; Bhatia, N.P. Sandhu. G.S., Johal M.S., 2002). In present study the LC_{50} values were 0.011, 0.015, 0.019 and 0.025 ml/liter at 24, 48, 72 and 96 hours. A review of literature revealed that the LC_{50} values of phosphamidon for *Channa punctatus* in Bundelkhand region was comparable to the estimated LC_{50} values of previous workers.

The abnormal changes in the behaviour of *Channa punctatus* may be due to the direct manifestation of the disturbances in the physiological mechanism (Fernando, M.D. and E.A. Moliner 1991; Barto B.A., and G. K., Iwama 1991; Wilson, R.W., Taylor E.W., 1993). Swimming movement, restlessness condition, loss of balance, jumping etc were observed in this study may be due to the effect of phosphamidon on central nervous system (CNS) (Brain 1967; Jai Nath and Rao 1984). The rapid breathing was observed when the fishes introduced in toxic environment of phosphamidon. This may be due to hyper excitability which involves considerable energy expenditure and there by making greater demands of oxygen. (Verma et al., 1979; Charistoforides C., & Hedley-Whyte J., 1969; Mishra C.K., Hakim A, Kumar J., 1994 ; Odiete W.O., 1999; Das, P. C., et al., 2003)

(b) Haematological and biochemical study:-

Phosphamidon is one of the exceptional organophosphorus pesticides comprehensively used in agricultural programme in all over the Asian countries. Indiscriminate uses of pesticides result in environmental pollution leading to accidental poisoning of different organism especially fishes in the ecosystem. Most of the chemicals are continuously added to the water bodies through run-off water from agricultural field, causing poisoning of fishes. The physiological stress caused by these chemicals can be studied by haematological parameters, as the actual health condition of an animal is reflected through the blood parameters. So the studies on haematological parameters are important for monitoring the fish health conditions. Phosphamidon impairs metabolic activities through tissue damage leading to reduced growth and health of organism. (Tripathi G., and S. P. Shukla 1988; Sailajaraghuram D., and B.N., Naidu 1989; Khangarot, B.S., et al., 1991; Reddy D.C., et al., 1992; Mukherji M. K. and S.K., Khonr 1984 ; Mathur K.K., 1991; Sastry K.V., and A., gupta 1994; Pulla et al., Mishra 1997) Hence it become clear again that haematological investigations are greatly helpful to provide the important informations about fish health. Stein J. E., et al., (1992); De La Torre et al., (2000) reported that the blood parameters of diagnostic importance are erythrocytes and leucocytes count. Haemoglobin and haematocrit are also responded to environmental factors such as toxicological stress due to water contaminants. Numerous workers have been reported the altered haematological and biochemical parameters to make a decision for effected biological activities and the harmful consequence of contamination due to phosphamidon and

other oraganophosphorus pesticides (Goel et al., 1986; Khangarot B.S., and P.K., Ray 1988e; Gupta. A. K., 1995; Poonmani R., and B., Dhanakkodi 1996; Jeba kumar S.R.D. et al., 1997; Sastry et al., 1997; Imbamani N., and R., Shrivassan 1998; Rajamannar K., and L., Manohar. 1998; Lata et al., 2001; Patnail et al., 2002).

The haematological and biochemical data's are presented in table 09 and 10 separately for acute and chronic toxicity tests. The table shows that the reduction was originated in values of haemoglobin percentage (Hb%), TEC and PCV behind every part of exposure periods to lethal and sub lethal concentrations of phosphamidon. The decreased response in figures of Hb% and PCV were positively correlated with TEC. The anamic response may possible due to the inhibition of erythrocytes production. (Shekhar P., and I., Christy 1996; Anand Kumar, A. and Tripathy N.K. 2001; Chandra et al., 2001) Haemoglobin is synthesized in the body of an organism from protein and iron. Liver is the store house of iron in the body. Phosphamidon is known to bring about necrotic changes in the liver of the fishes (Verma et al., 1981). This might has led to non availability of enough iron in the body of exposed fishes leading to a decrease in Hb% which in turn decreases the oxygen carrying capacity of the blood (Chandshekhar S., and N. Jayabalan 1993; Anand Kumar, A. and Tripathy N.K. 2001). The behavioural responses also confirm the low O_2 consumption.

Table 09 and 10 shows that the levels of TLC and ESR increased after all exposure periods of phosphamidon. The level of TLC increased because leucocytes are involved in the regulations of immunological function of body (Santa kumar et al., 2000). An

increased in TLC thus occurs as a protective response to stress (Das R., 1998). Hence it become clear that TLC and ESR have negative correlation with TEC. (Goel K A., and Maya 1986; Kumar B., Benerjee V., 1990; Singh S. and Bhati D.P.S., 1991). Increased level in the values of ESR was also observed by Gupta et al., (1995); Singh and Bhati (1991); Mala F.A., G. Sharma (2009)..

The reduction in the values of Hb%, TEC and PCV indicates that the fishes suffer from anaemia or heamodilution. The alterations in MCV, MCH, and MCHC were also observed in comparison to control fishes, because they are interrelated with haemoglobin, TEC and PCV. Gradually falling in values of MCV, MCH and increased MCHC values were observed in the present study during acute and chronic toxicity conditions. These alterations verify that the fishes are suffering from anaemia during exposure periods due to iron deficiency and damaged tissue conditions especially liver. Similar haematological effects of phosphamidon in fishes, after various exposure periods have also been reported by many bio researchers (Chaudhry D.K., et al., 1984; Anand Kumar A., and Tripathy N.K., 2001; Bhatia et al., 2002; Das B. K., and S.C., Mukherjee 2003).

The biochemical parameters (blood glucose) is also an important indicator to study the internal environment of fishes. A significant increase was observed in blood glucose level intoxication of phosphamidon after acute and chronic exposure periods (Table 09 and 10). The change in blood glucose may be due to stress in fishes (Nataragan G.M., 1989; Shobha R.J.V., et al., 1989; Chandrasekhar and Jayabalan S., 1990; Sikoki, F. D., & Enajekpo, H.O.S., 1991; Chandshekhar S. and N., Jayabalan 1993; Das B.K., and S.K.,

Mukharjee 2000; Luskova et al., 2002; Saufy et al., 2007). The stress related hyperglycemia was reported in many species of teleost. It was noticed that cortisol has shown to promote catabolism of peripheral tissue via gluconeogenesis, leading to hyperglycemia. Increase in blood glucose might have resulted from gluconeogenesis to provide energy for the increased metabolic demands imposed by insecticides stress (Ghosh T. K., 1989; Jyothi B., and G., Narayan 1999; Kumar Hemant and Gupta A.B., 1997).

The depletion of glycogen content in liver and muscles by the effect of pesticides on fishes was also observed by many workers (Srivastava A.K., 1981; Nemcsok and Bones 1982; Singh H.H., Srivastava A. K. 1982; Nataragan G. M., 1989; Luskova V., et al., 2002; Medda et al., 2003). The impaired carbohydrate metabolism may also causes hyperglycemia as shown in the present study both in acute and chronic exposure of phosphamidon (Chandshekhar S. and N., Jayabalan 1993Kumar Hemant and Gupta A.B., 1997).

(c) Study of seasonal variation:-

The data obtained in figure 23-31 and 32-40 showed that haemoglobin percentage, TEC and PCV were decreased but TLC, ESR and blood glucose level were increased following exposure to phosphamidon from untrited fishes both during acute and chronic experiment. The maximum values of Hb%, TEC, TLC, and PCV were observed at the end of rainy season and lowest values of parameters were found in strong summer season. The average values of all the parameters in control fishes were found in winter season. This could be due to the process of haemoconcentration occurs during the late

spring and summer followed by haemodilution in the late summer. The highest blood glucose level was found in rainy season. It indicates that the low temperature and rich food supply stimulate metabolism in fish body. The values of MCV, MCH and MCHC were also fluctuated according to the levels of Hb%, TEC, and PCV. The lower activity of all the parameters in summer season may also be due to the high temperature of water as shown in chapter 4, O_2 availability was also reduced in summer season (Schaefer A.A., 1925; Kaplan and Carousa 1956; Raizada et al., 1983; Harding J., and Hogland L. B., 1984; Joshi P.C., 1989; Denton J.E., and Yousef M.K., 1995; Collazos et al., 1998) causes the decreased activity of TEC, HB%, and PCV.

Determination of LC_{50} for Phosphamidon on *Channa punctatus*

Table 06: First exploratory test

S.No.	Conc. ml/liter	No. of fishes	24 Hours		48 Hours		72 Hours		96 Hours	
			M	%M	M	%M	M	%M	M	%M
1.	0.002	5	0	0	0	0	0	0	0	0
2.	0.040	5	5	100	-	-	-	-	-	-

Table 07: Second exploratory test

S.No.	Conc. ml/liter	No. of fishes	24 Hours		48 Hours		72 Hours		96 Hours	
			M	%M	M	%M	M	%M	M	%M
1.	0.007	5	0	0	0	0	0	0	1	20
2.	0.014	5	0	0	1	20	1	40	2	80
3.	0.021	5	2	40	2	80	1	100	-	-
4.	0.028	5	4	80	1	100	-	-	-	-

Table 08: Definitive test

S.No.	Conc. ml/liter	No. of fishes	24 Hours		48 Hours		72 Hours		96 Hours	
			M	%M	M	%M	M	%M	M	%M
1.	0.008	10	0	0	0	0	1	10	2	30
2.	0.010	10	0	0	0	0	2	20	2	40
3.	0.012	10	0	0	1	10	2	30	3	60
4.	0.014	10	0	0	2	20	2	40	3	70
5.	0.016	10	1	10	2	30	3	60	3	90
6.	0.018	10	2	20	2	40	3	70	3	100
7.	0.020	10	3	30	4	60	3	90	1	100
8.	0.022	10	4	40	4	80	2	100	-	-
9.	0.024	10	6	60	4	100	-	-	-	-

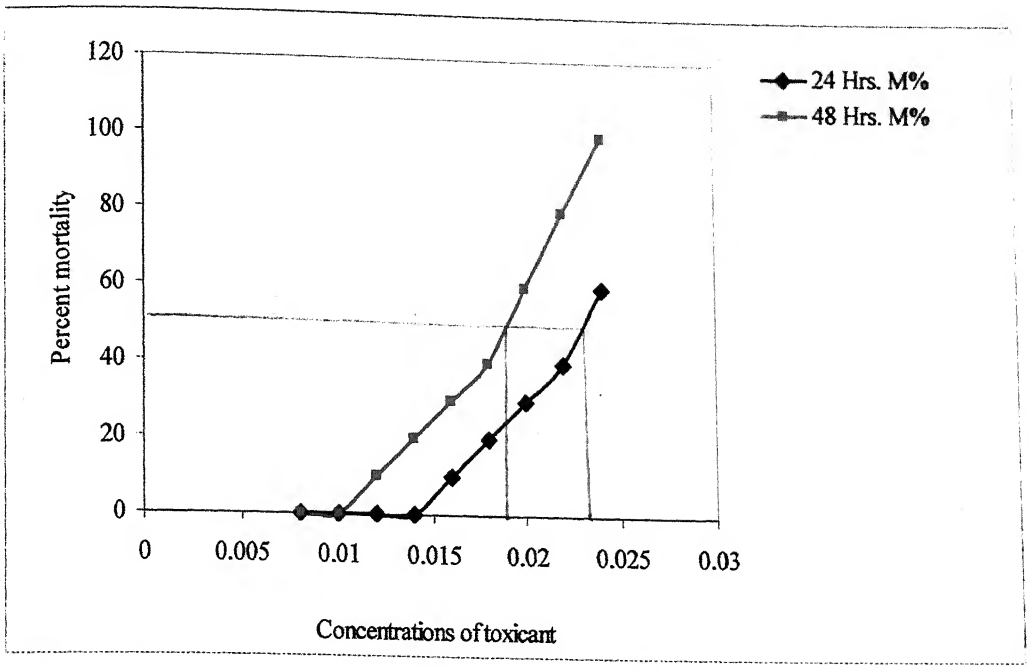


Fig 21: LC_{50} of Phosphamidon after 24 hours and 48 hours

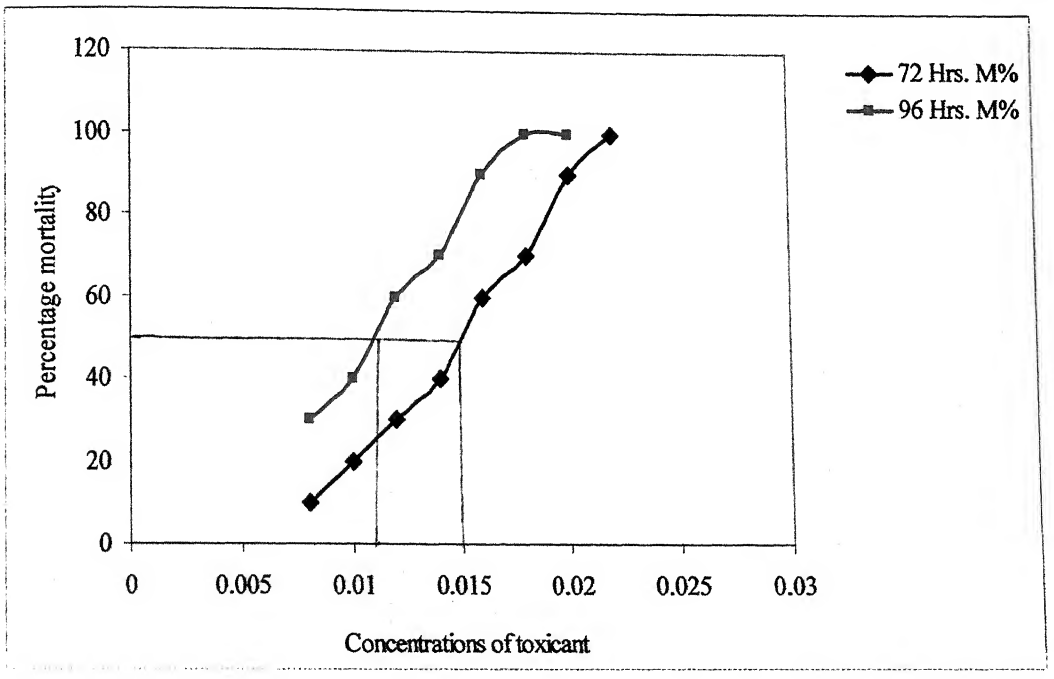


Fig 22: LC_{50} of Phosphamidon after 72 hours and 96 hours

Table No. 09

Effect of acute toxicity of Phosphamidon on selected blood parameters in fresh water fish *Channa punctatus*

S No.	Parameters	Control	Exposure Period			
			24 Hours	48 Hours	72 Hours	96 Hours
01.	Hb (g/100ml)	14.1 ±0.72	10.9** ±0.63	11.5** ±0.10	11.1** ±0.49	11.5** ±0.36
02.	TECx10^6/mm^3	3.85 ±0.07	3.42** ±0.05	3.45** ±0.07	3.48 ±0.03	3.49** ±0.04
03.	TLCx10^3/mm^3	3.2 ±0.32	4.0* ±0.20	4.2* ±0.47	4.2* ±0.40	4.3* ±0.65
04.	ESR (mm)	2.5 ±0.11	3.9 ±0.15	4.1 ±0.34	4.1 ±0.23	3.8* ±0.55
05.	PCV%	33.33 ±3.05	22.33* ±3.05	24.33* ±3.50	25.66* ±3.51	26.66* ±3.21
06.	MCH pg	36.66 ±1.34	31.55* ±1.53	33.30* ±0.43	31.26* ±1.37	33.10* ±1.03
07.	MCHC %	42.51 ±2.06	49.33 * ±4.24	47.80* ±6.60	44.11* ±7.10	43.56* ±5.54
08.	MCV um^3	86.82 ±7.86	64.45* ±10.65	70.57* ±9.73	75.85* ±0.48	76.86* ±9.63
09.	Glucose (Units)	64.73 ±5.60	70.07* ±6.53	73.71* ±6.45	73.12* ±4.67	74.91* ±4.93

* - Significant at P < 0.01; ** - Significant at P < 0.001

Table No. 10

Effect of chronic toxicity of Phosphamidon on selected blood parameters in fresh water fish *Channa punctatus*

S No.	Parameters	15 Days		30 Days		45 Days	
		C	T	C	T	C	T
01.	Hb (g/100ml)	13.9 ±0.60	11.9** ±0.36	14.6 ±0.57	12.5* ±0.05	14.3 ±0.66	12.9 ±0.15
02.	TECx10^6/mm^3	3.77 ±0.11	3.50* ±0.09	3.80 ±0.90	3.57* ±0.08	3.88 ±0.09	3.63* ±0.08
03.	TLCx10^3/mm^3	3.1 ±0.40	4.0* ±0.37	3.2 ±0.35	4.2 * ±0.35	3.5 ±0.40	4.8** ±0.10
04.	ESR (mm)	2.3 ±0.20	3.6 ** ±0.11	2.3 ±0.15	3.8* ±0.75	2.4 ±0.55	4.0 ±0.62
05.	PCV%	27.66 ±2.88	19.66* ±2.08	29.66 ±3.05	21.00* ±2.64	33.00 ±2.00	23.33** ±2.30
06.	MCH pg	36.82 ±0.48	34.93* ±1.68	36.97 ±0.51	35.08* ±0.94	36.92 ±0.90	35.63* ±0.88
07.	MCHC %	49.89 ±4.56	60.82* ±4.67	47.76 ±5.43	60.29* ±7.36	43.48 ±1.89	55.80* ±5.70
08.	MCV um^3	73.34 ±7.61	52.75* ±7.40	75.39 ±9.72	58.75* ±7.16	85.04 ±4.69	64.25* ±5.68
09.	Glucose (Units)	60.46 ±8.35	70.87* ±9.14	63.55 ±8.67	76.03* ±9.48	66.03 ±5.98	76.92* ±9.47

* - Significant at $P < 0.01$; ** - Significant at $P < 0.001$

Graphical comparison of seasonal variation in acute toxicity experiment

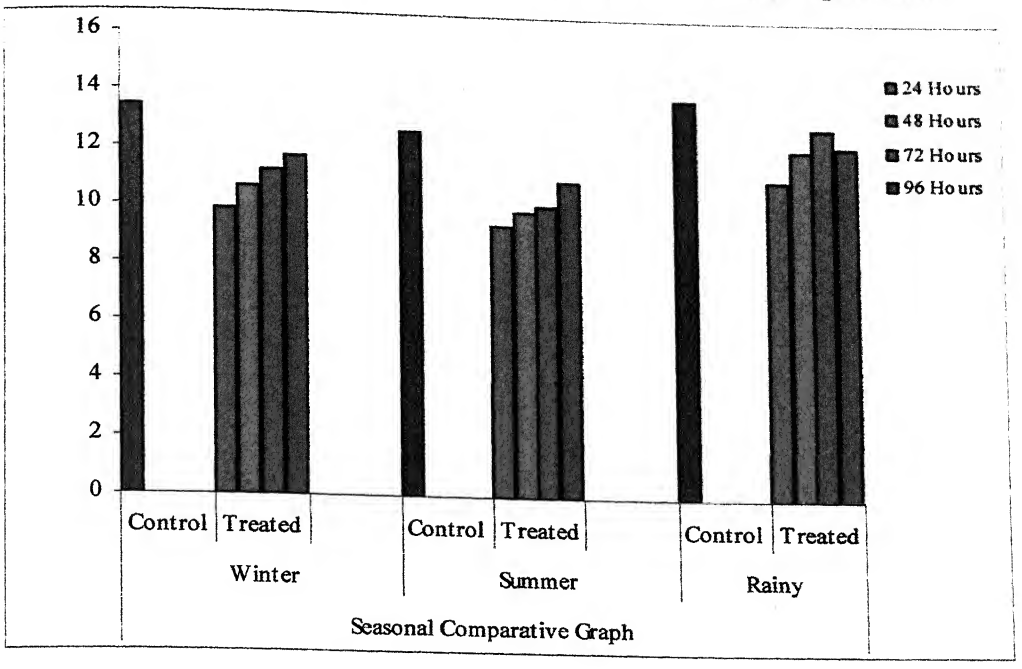


Figure 23. Haemoglobin

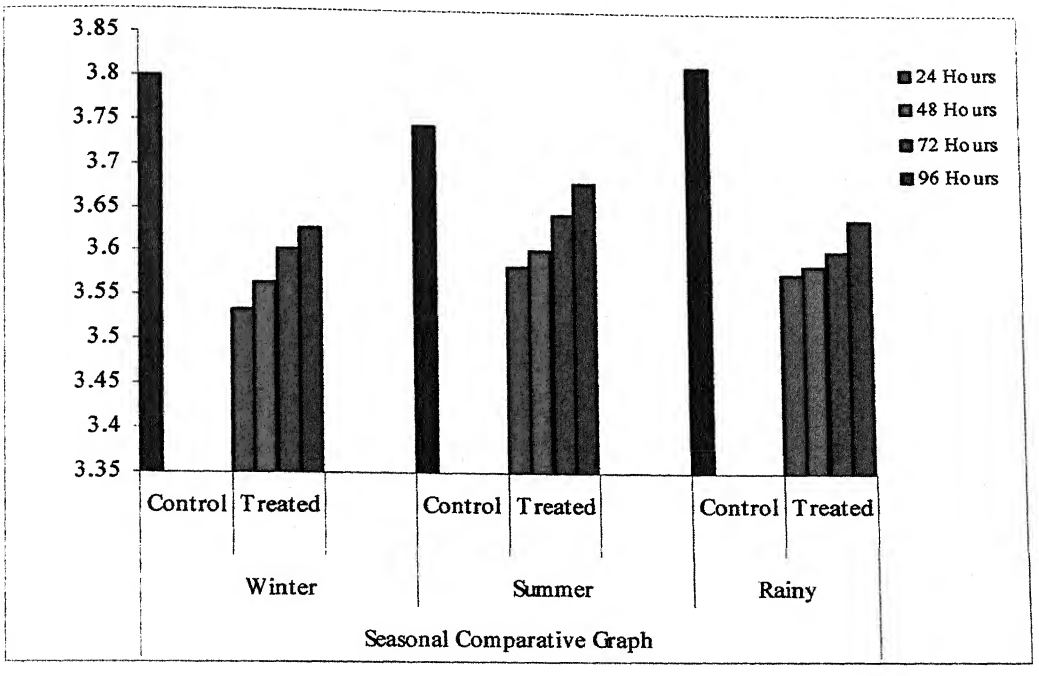


Figure 24. TEC

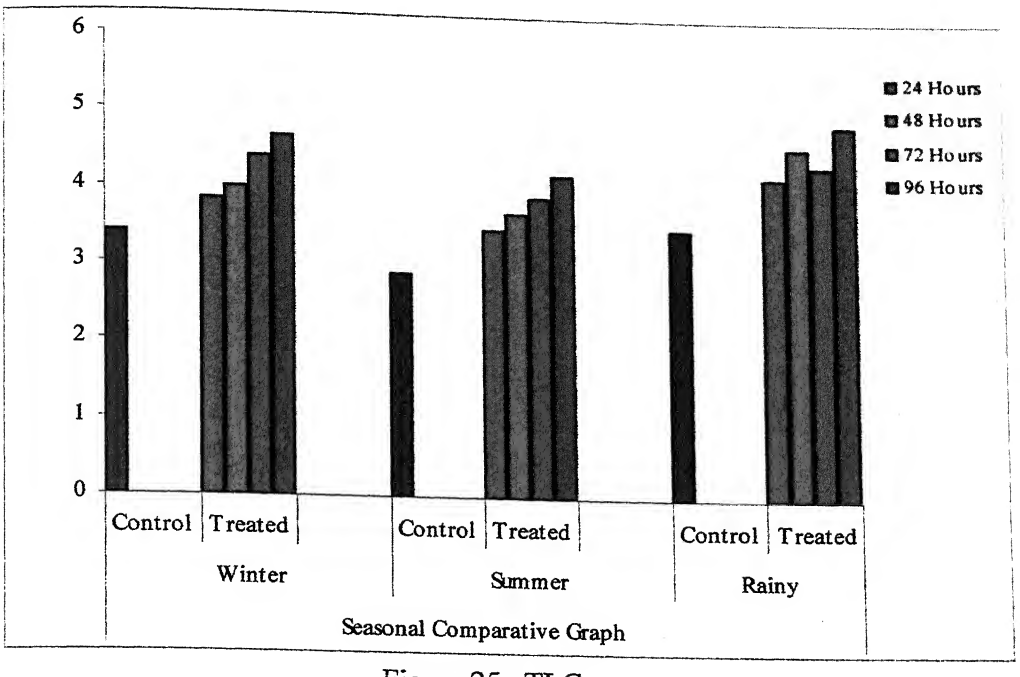


Figure 25. TLC

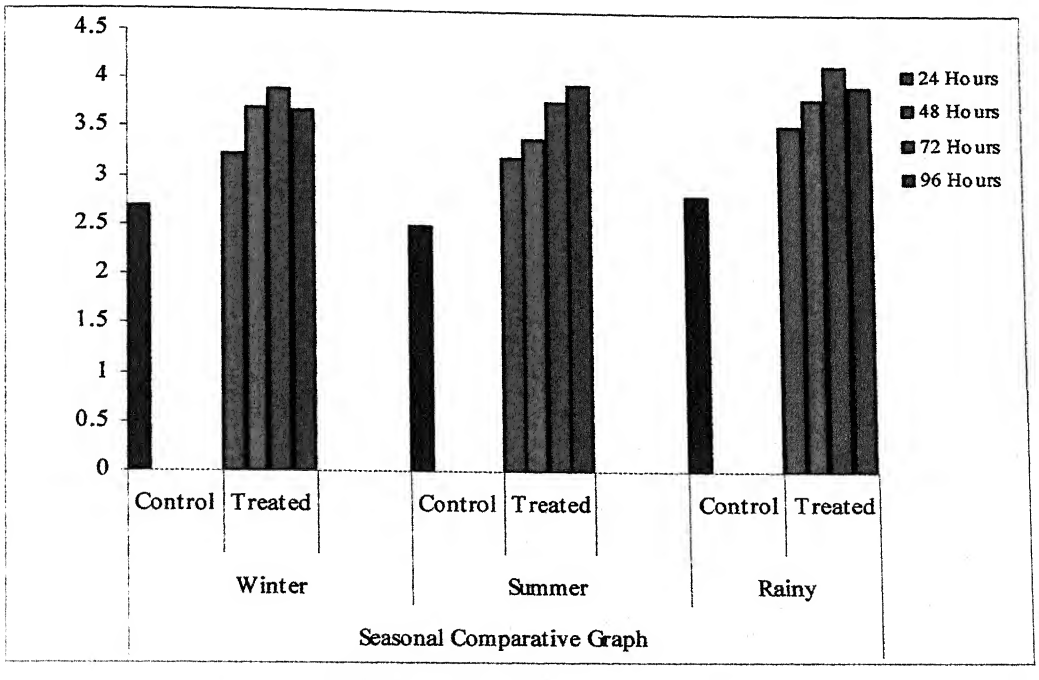


Figure 26. ESR

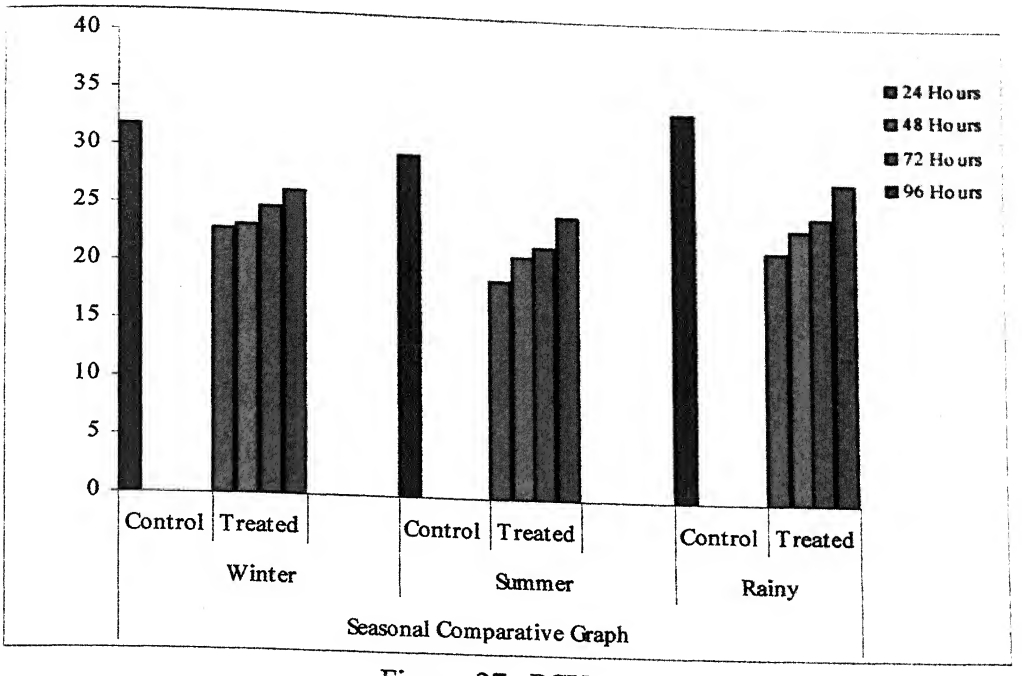


Figure 27. PCV

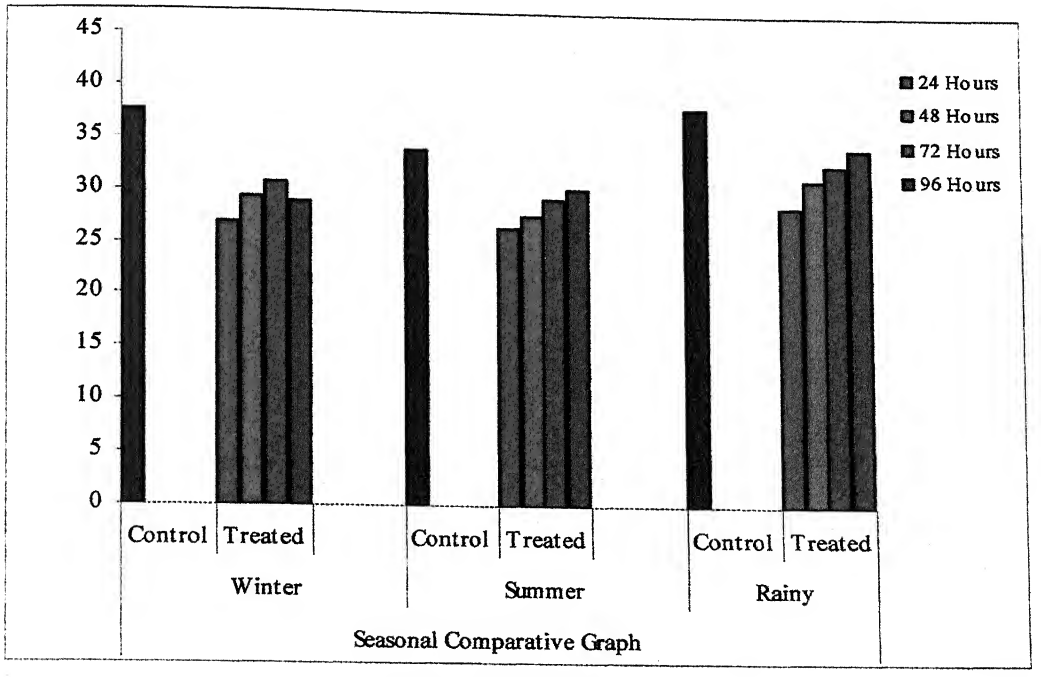


Figure 28. MCH

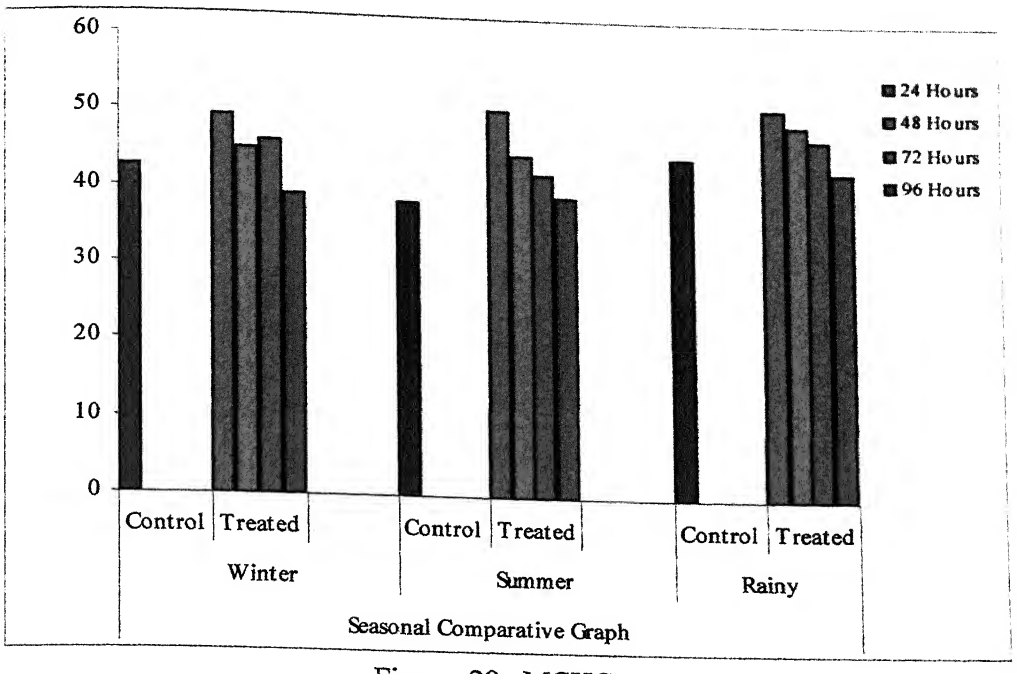


Figure 29. MCHC

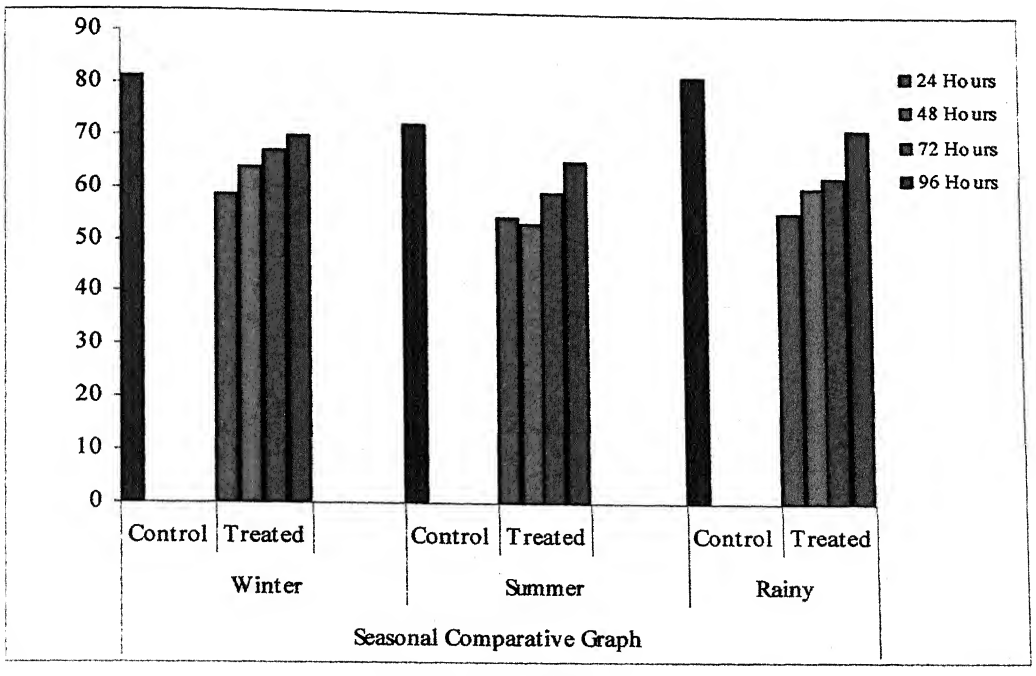


Figure 30. MCV

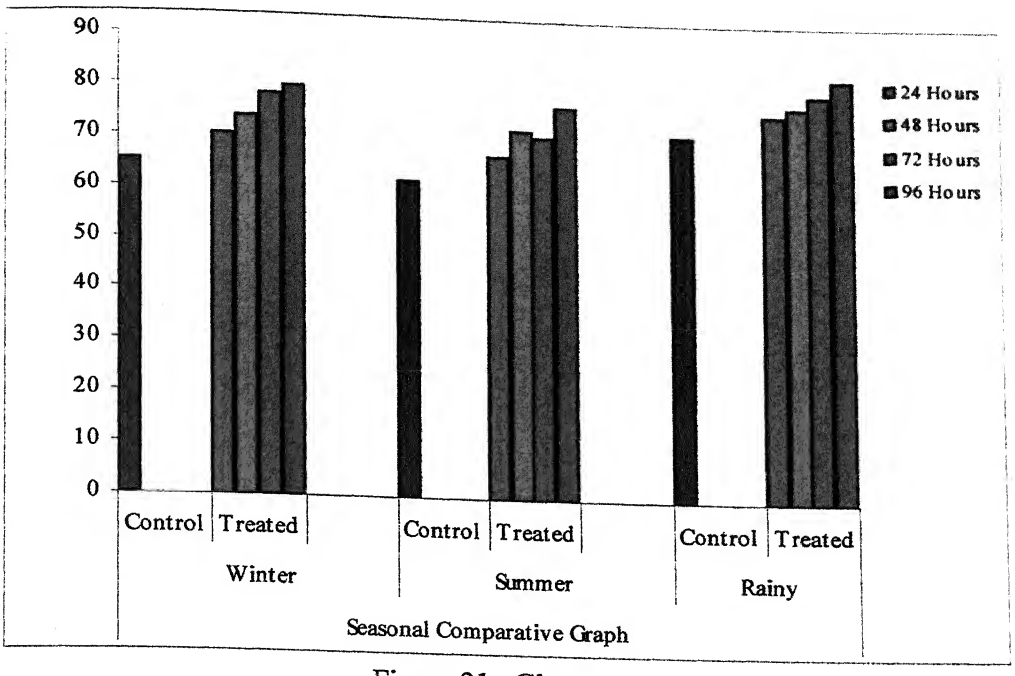


Figure 31. Glucose

Graphical comparison of seasonal variation in chronic toxicity experiment

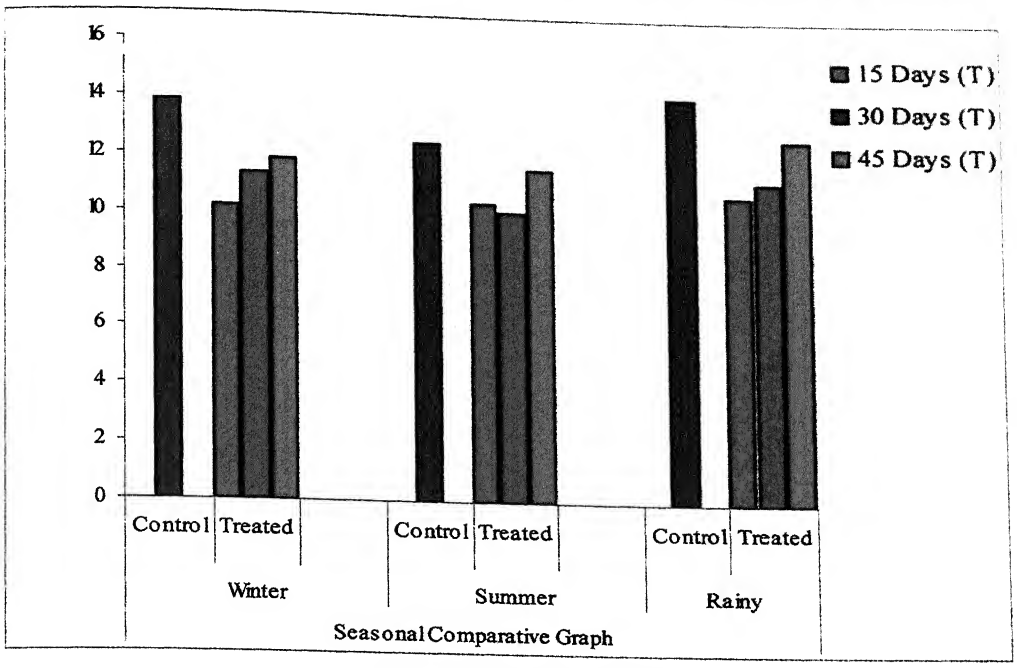


Figure 32. Haemoglobin

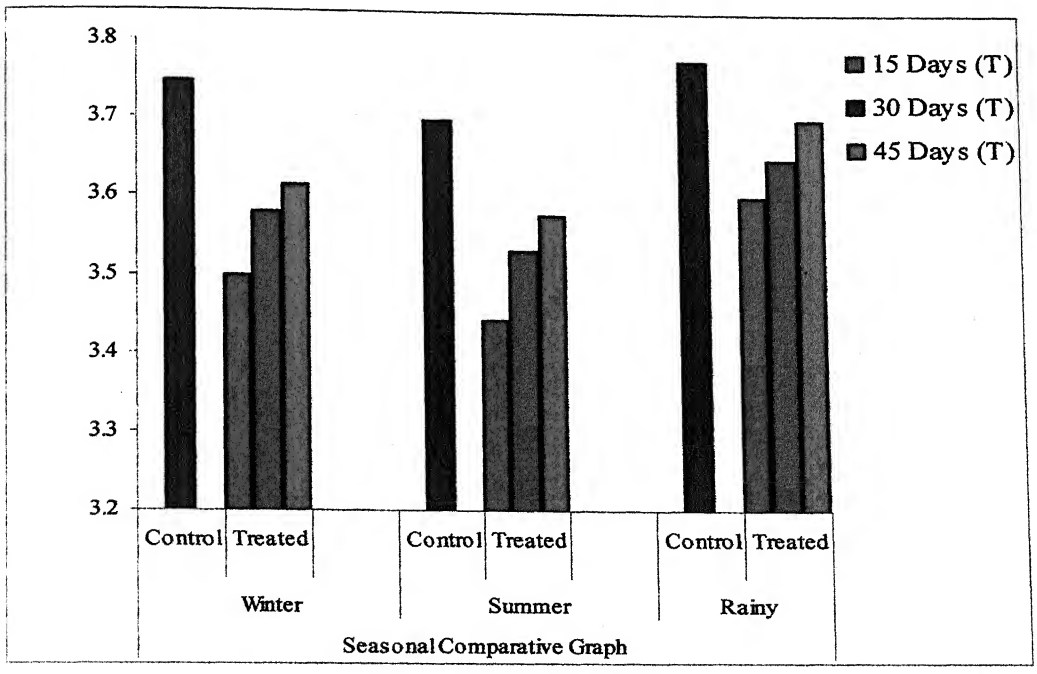


Figure 33. TEC

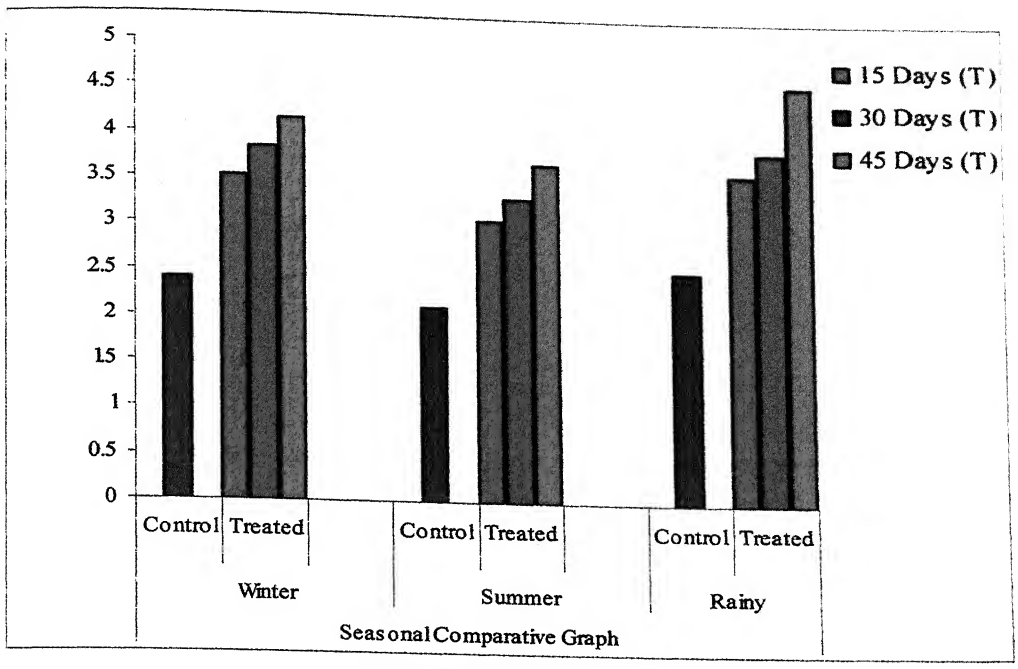


Figure 34. TLC

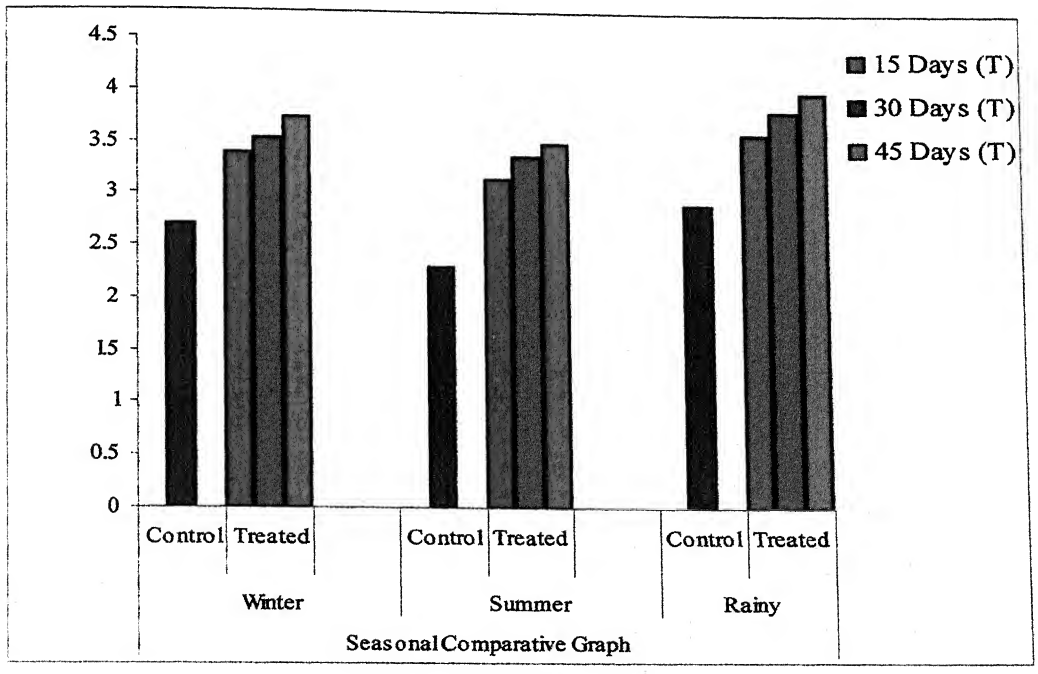
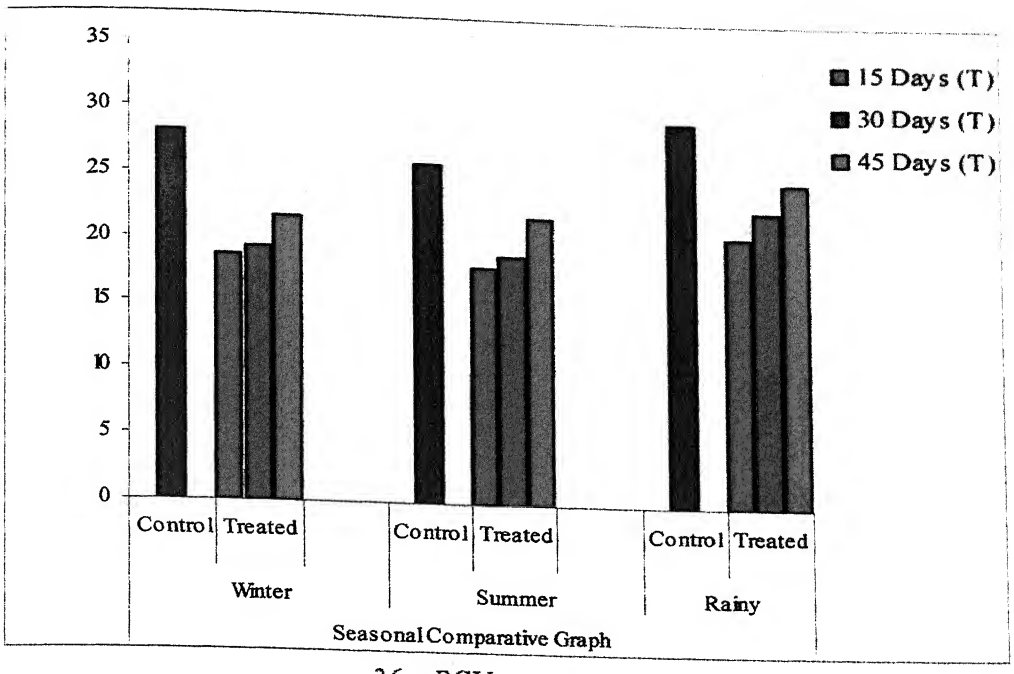


Figure 35. ESR



36. PCV

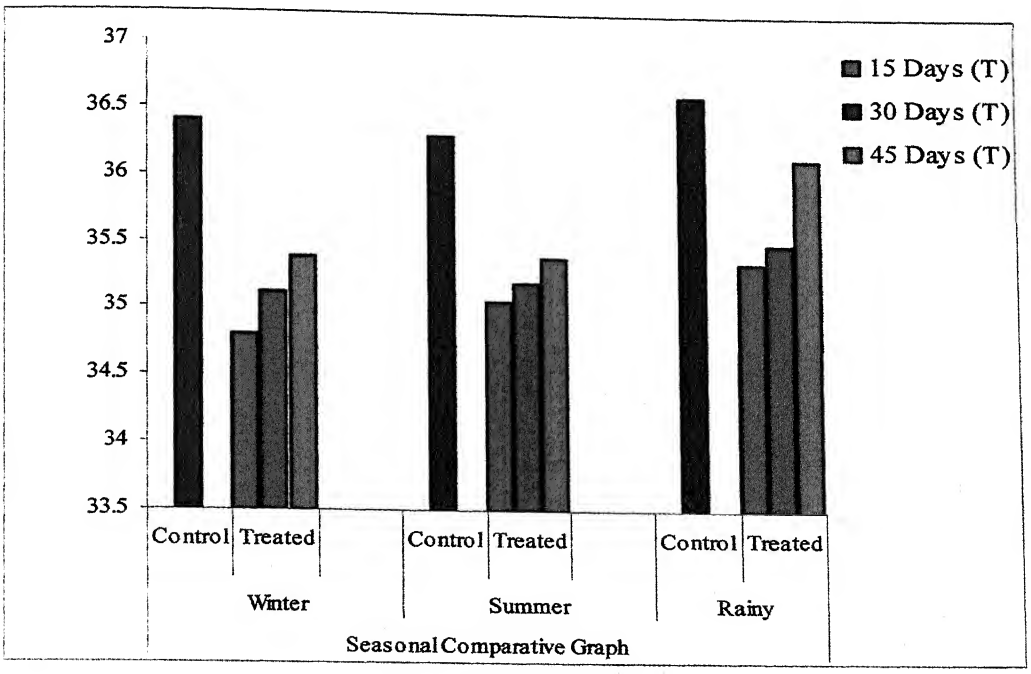


Figure 37. MCH

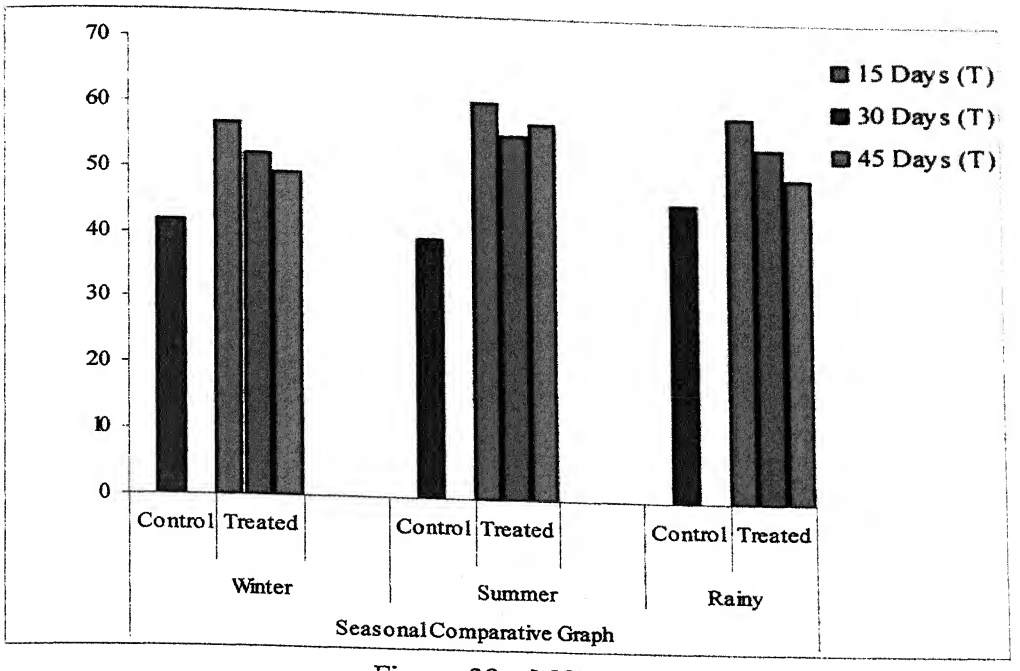


Figure 38. MCHC

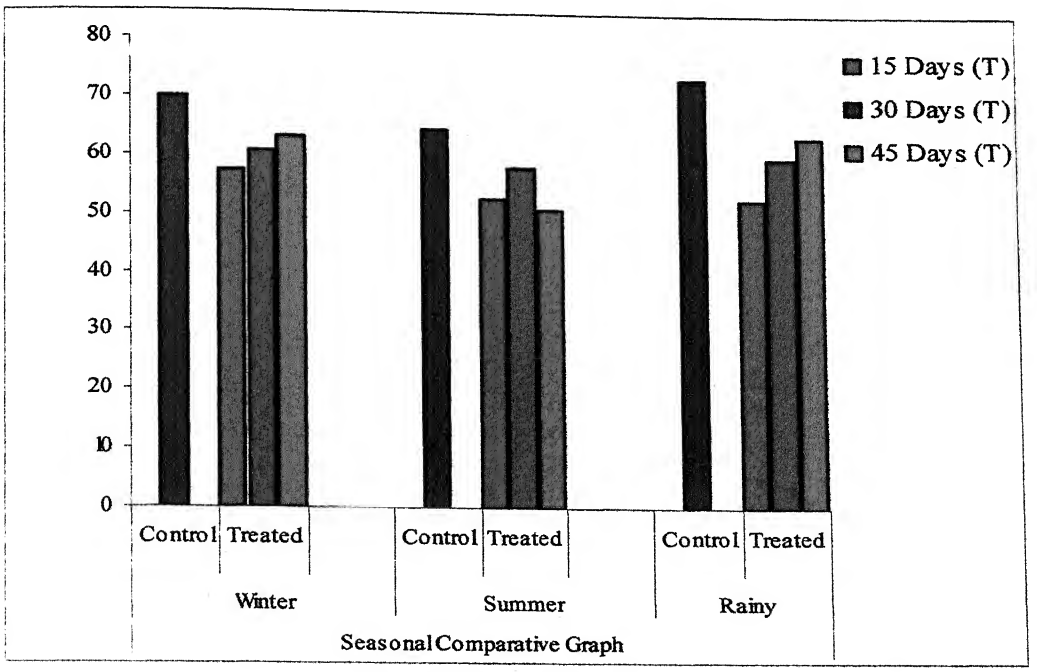


Figure 39. MCV

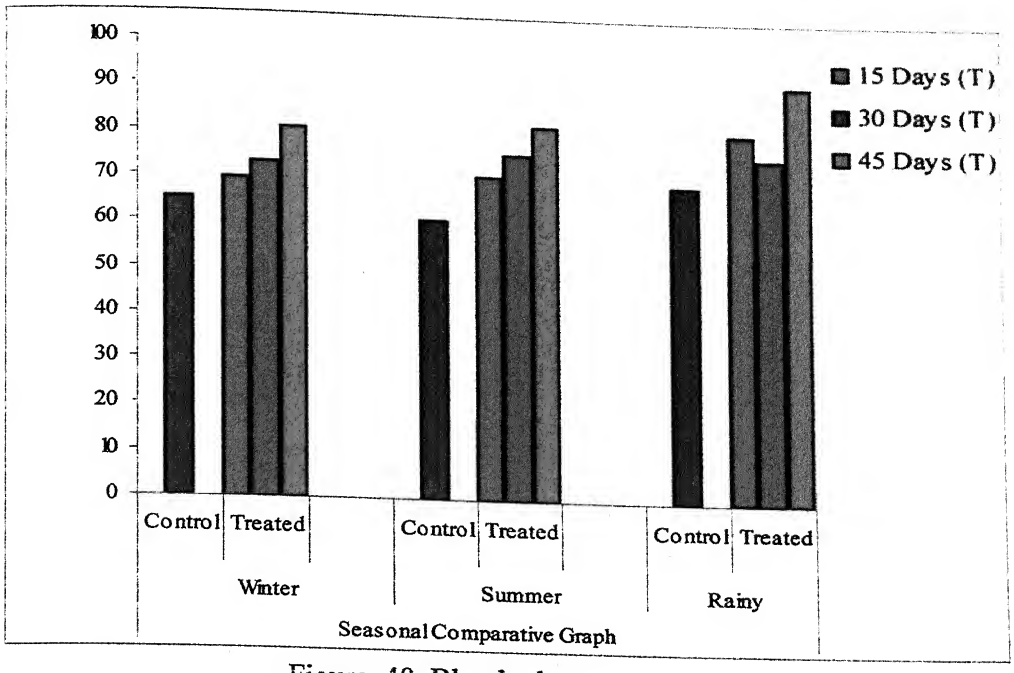


Figure 40. Blood glucose

Chapter - 7

EFFECT OF METASYSTOX ON BLOOD PARAMETERS OF *CHANNA PUNCTATUS*

EFFECT OF METASYSTOX ON BLOOD PARAMETERS OF *CHANNA PUNCTATUS*

Metasystox 25% E.C. was a systemic organophosphorus insecticides used against many insects. It is a water emulsifier insecticide. The chemical composition of this compound is Oxydemeton-methyl, *S*-[2-(ethylsulfinyl) ethyl] *O*, *O* dimethyl. It is manufactured by Bayer India limited, Mumbai.

Results

(a) Acute toxicity bioassay:-

To find out the accurate determination of LC_{50} values, two exploratory and a definitive test were conducted. In first exploratory test 100% mortality was observed in 0.050 ml/l of metasystox while no mortality in 0.010 ml/l (table 11). In second exploratory test four ascending series of concentrations (0.015, 0.025, 0.035, and 0.045 ml/liter) were taken to get narrow range of concentrations (table 12). In second range finding test 100 % mortality occurred at 0.045 ml/l after 24 hours exposure period but 20 % mortality was seen at 0.015 after 96 hours exposure of metasystox. Then seven concentrations were selected for definitive test (table 13) on the basis of second exploratory test and record the percent mortality of each selected concentration. Now a curve was plotted between percent mortality and concentrations of metasystox and sketch a line intersecting through the concentrations and 50% mortality level (fig no. 41 and 42). The LC_{50} values determined by graphical method were found to be 0.034, 0.030, 0.026 and 0.022 ml/l for metasystox at 24, 48, 72 and 96 hours.

The effect of metasystox on fish behaviour has also been observed in *Channa punctatus*. Initially the fish gives the impression of restlessness, lethargy, rolling movement and lost their balance and natural behaviour. After some time they showed erratic and burst swimming with augmentation in respiratory frequency swimming near the water surface for oxygen uptake. Toxicant exposed fish also secretes excessive mucous in comparison to control fishes. Under higher concentrations they showed hemorrhagic patches on their skin.

(b) Haematological & biochemical study:-

The haematological & biochemical parameters of control and metasystox exposed fishes at LC_{50} concentrations are presented in table 14. The Haemoglobin percentage and total erythrocytes counts (TEC) were increased significantly after 24, 48, 72 and 96 hours of exposure periods at $P < 0.01$ and $P < 0.001$. The haemoglobin percentage in control fishes was 14.1 gm% where as in treated fishes it was 16.1, 16.2, 16.3 and 16.5 gm% at the end of 24, 48, 72 and 96 hours respectively. The Hb % level was low at 24 hours of LC_{50} and high in 96 hours exposure. Although TEC was also more than control fishes but it was gradually increased with increasing exposure periods. A decreased level was observed in TLC and ESR. The difference was statistically significant at $P < 0.01$ and $P < 0.001$. The ESR was lower in 24 hours but it was also gradually increased with running exposure periods.

The values of PCV, MCH and MCV were significantly increased at $P < 0.01$ after 24, 48, 72 and 96 hours exposures. The values of PCV and MCV for 96 hours were also significant at $P < 0.001$ and same

case was found in value of MCH during 72 hours exposure period. In case of MCHC, it was decreased significantly at $P < 0.01$. The table 14 showes that the levels of glucose increased significantly at $P < 0.01$ in treated fishes. The value of blood glucose was 63.46 in control fishes where as fishes exposed to acute toxicity concentrations of metasystox showed 68.36, 73.05, 74.58 and 76.14 units at 24, 48, 72 and 96 hours respectively.

The haematological & biochemical parameters of control and metasystox exposed fishes at 1/10 of 96 hours LC_{50} concentrations are presented in table 15. The level of Hb%, TEC, PCV, MCH and MCV were significantly increased at $P < 0.01$ in treated fishes than untreated ones after the end of 15 days, 30 days and 45 days intoxication of metasystox. The level of TEC, PCV and MCV were lesser during 15 days exposure but gradually increased with increasing exposure periods. A significant decreased level of TLC, ESR and MCHC were observed at $P < 0.01$ and $P < 0.001$. The table 15 also indicates the change in blood glucose level in *Channa punctatus*. The blood glucose level was increased significantly at $P < 0.01$ in the insecticidal treated fishes.

(C) Study of seasonal variation:-

The seasonal changes of certain haematological and biochemical parameters (glucose levels) are presented in the figure 43-51 during acute toxicity bioassay and chronic toxicity bioassay was presented in fig 52-60. In toxicity test TEC and PCV were decreased during warm season in unexposed fishes. The Hb%, TEC, and MCH were lower in late summer season, while higher in beginning of rainy

season in control fishes. The value of TLC and ESR were slightly altered during summer and rainy seasons. Although Hb% TEC, PCV, MCH and MCV increased and other parameters were decreased in treated fishes as shown in table 14, but the activity levels of all the parameters were low in summer season and high in rainy season. In case of PCV maximum value was observed in winter season in control fishes. However PCV in treated fishes was almost similar during rainy and winter season but average in summer season. The levels of MCH, MCHC and MCV were also observed. They were also fluctuated during different seasons according to the values of TEC, Hb% and PCV. The highest blood glucose levels were observed during rainy season. In chronic experiments Hb%, TEC, TLC and ESR were slightly low in late summer season. In treated fishes the levels of Hb%, TEC and PCV were moderately change and low in summer seasons comparably. The levels of MCH, MCHC and MCV were also observed during chronic exposure. They were also rise and fall during different seasons according to the standards of TEC, Hb% and PCV. The blood glucose level was higher in rainy season in control fishes while in treated fishes the highest value was observed after 45 days of exposure period.

Discussion

(a) Acute toxicity:-

Indiscriminate use of insecticides has elevated the risk of contamination of environment and aquatic habitat. Various bioresearchers have been reported the LC_{50} values of metasystox in

different fish species. Natrajan G.M., (1983) notified the LC_{50} value 0.5 mg/l at 48 hours in *Channa striatus,* while Jhon et al., (1989) reported 12.9 ppm LC_{50} for 96 hours in *Heteropneustes fossilis*. Several workers have also been reported the toxicity of metasystox and other organophosphrous insecticides (Mahajan and Juneja 1979; Dhilon and Gupta 1983; Natrajan G.M., 1983; Ghosh and Chatterji 1989;Valicre E.J. and C.J., Stickney 1989; Meenaxi Das and Sambhu Prasad 1996).

Kumar Hemant and A. B., Gupta (1997) estimated the LC_{50} values in *Heteropneustes fossilis*. According to them the LC_{50} were 29.5, 24.50, 17.00 and 17.00 ppm for 24, 48, 72 and 96 hours respectively. In the present study the LC_{50} values were 0.034, 0.030, 0.026 and 0.022 ml/liter at 24, 48, 72 and 96 hours respectively, which correlates the LC_{50} values of other workers. A review of literature showed that, aquatic environment is polluted by the use of metasystox so the present study is aimed at investigating the effect of metasystox on blood parameters of affected organisms like fishes of Bundelkhand region.

Numerous abnormal behaviours such as impression of restlessness, rolling movement and lost their natural behavior and balance, erratic swimming with increment in respiratory frequency, swimming near the water surface for oxygen uptake observed after exposure period. Comparable changes in behavior of fresh water fishes on exposure to different organophosphorus toxicants were reported by several biologists. (Salim Mustfa and Ajmal Murad 1983; Kumar Hemant and A.B., Gupta 1997; Roopa Methew et al., 1997; Sadhu et al., 2001; Vatukuru S.S., 2005). These changes could be due

to the effect of metasystox on the central nervous system. Increased opercular movement of fishes when introduced in toxic media implied rise in oxygen consumption. This also may be due to hyper excitability which involves energy expenditure and producing greater demand of oxygen. Behavioral changes resulting in restlessness and loss of balance erratic swimming and hemorrhage have also been observed in the fishes by other workers (Cleveland et al., 1972; Jai Nath and Rao 1984; Beitinger T. L., 1990; Little E. E., and S. E. Finger 1990; Little et al., 1993 b; Allin C.J., and R.W., Wilson. 2000; Brewer et al., 2001; Singh Snehlata, Sadhu D. N., 2001; Vatukuru S. S., 2005).

(b) Haematological and biochemical study:-

Organophosphorus insecticides have been widely used by farmers to control insects, pests and disease vectors. They ultimately find their way into aquatic habitats such as river, lakes and ponds. They become highly toxic and ultimately disturb the food chain. The haematological and biochemical studies are useful in assessing the health of fish subjected to changing in environmental conditions. The pesticides play an important role to alter the blood parameters of fishes to a considerable extent. Keeping this in view the study was conducted to assess to the changes in haematological and biochemical parameters in fishes (*Channa punctatus*) exposed to lethal and sub lethal concentrations of metasystox. Many workers have been contributed the study of haematological changes due to organophosphorus pesticides in fishes (Agrawal S. J., and A. K., Srivastava 1980; Jagdish Mishra and Anil K., Shrivastava 1981; Singh B. B. & Narain, A. D.,1982 Pandey et al., 1980; Balint et al., 1995; Edsall, C. C., 1999; Nath. Ravindra and Banerajee V., 1999).

Haematological data are presented in table 14 and 15. Elevations in Hb%, TEC, PCV were significant at $P < 0.01$ and $P < 0.001$ after the end of all exposure period. Several bioresearches have mentioned the similar reports of Hb%, TEC and PCV using metasystox during acute and chronic toxicity exposure (Mahajan and Juneja 1979; Dhilon and Gupta 1983; Natrajan G.M., 1983; Valicre E.J., and C.J., Stickney 1989; Meenaxi Das and Sambhu Prasad 1996; Josiph P., Jhon 2007). In the addition metasystox can cause the low pH value during and chronic exposure period, so that the increased value of Hb%, TEC and PCV may be possible due to low pH value (Valicere E.J., 1989).

In the study with brook trout exposure to sub lethal conc. of cupric chloride also found significant increased in Hb% and PCV content Mitchell (1987) and Natrajan G. M., (1983) also showed the increased level of Hb%, TEC and PCV. It has been suggested that these haematological disturbances are a haemopoietic or erythrocytic mobilisation response caused by stress (Goel, K. A., 1982; Adams S.M., 1990; Barton B.A., and G.K., Iwama 1991; Wilson R.W., Taylor, E.W., 1993; Stein et al., 1992; Mikko Nikinmaa and Virpi Tervonen 2003; Vander et al., 2003).

In many species of fishes, the spleen is the primary storage site of erythrocytes (Gallaugher and Farrell 1998). Liberation of erythrocytes from the spleen is under adrenergic nervous or hormonal control. Thus, erythrocyte liberation can occur very rapidly, and is known to take place as a response to both exercise and hypoxic condition (Mahajan and Juneja 1979; Yamamoto et al. 1980, Yamamoto et al. 1983). The pioneering study by Swift and Lloyd (1974) showed that in acute hypoxia the urine flow rate of rainbow

trout increased simultaneously with an increase in blood haematocrit value. The increased excretion of water causes a reduction in plasma volume, as is observed in rainbow trout (Mahajan and Juneja 1979; Valicere E.J. ,1989; Mikko Nikinmaa and Virpi Tervonen 2003). A decrease in plasma volume will increase the number of erythrocytes per unit volume.

The level of TLC were decreased after acute and chronic toxicity bioassay agrees with the report, that the release of epinephrine during stress condition causes a decrease in the number of leucocytes counts, which show the weakening process of immune system (Alkaham et al., 1998; Ajani F., 2008; Khalid et al., 2008; Natrajan G.M., 1983).

Alkahem et al., (1998) and Ajani F., (2008) also showed the decreased no. of leucocytes after being exposed to organophosphorus pesticides. This is attributed to the reduction in the no. of lymphocytes Maule and Schrecr (1990) also noted the stress and level of cortisol affect the number of lymphocytes in immune organs.

In present study increased level of MCH, and MCV were observed while MCHC were decreased after the end of all exposure periods with metasystox. These alterations confirm polycythaemia in fishes due to stress condition (Goel K.A., 1982; Khattak I.U.D., Natrajan G.M., 1983). Another reason in alterations in haematological parameters in the lethal and sub lethal concentration of metasystox apper to due to damage respiratory epithelium. Mucus is also lost from the gills which causes the adverse effect in the absorption of O_2 (Natrajan G.M., 1983). Similar observation in depletion of O_2

consumption was made by Rath S., and Mishra B.N. (1980). Inhibited respiration was also observed by other workers using other organophosphorus insecticides (Hiltibran R. C. 1974; Kawatski J. A., and Mc. Donald M. J. 1974; Ranke B., and Rybicke 1975; Rath S., and Mishra B.N. 1980). The low rate of O_2 consumption caused hypoxic condition which is turn increase Hb% and TEC.

To study the health of fishes biochemical parameters are important indicators. (Jagdish Mishra and Anil K., Shrivastava 1981; Mishra, J., Shrivastava A. K., 1983; Edsall C.C., 1999; Vdesek J., et al., 2009). Changes in the biochemical parameters cause changes in metabolism due to the effect of various toxicants. Changes in the activity of several enzymes and carbohydrate metabolism have been observed by several workers (Hochachka et al., 1978; Srivastava A.K., 1981; Nemcsok and Bones 1982; Singh H.H., Srivastava A. K. 1982; Ghosh T.K. ,1989; Natarajan G. M., 1989; Kumar Hemant and Gupta A.B.1997; Luskova V., et al., 2002; Saufy H., et al., 2007). The blood glucose level in Indian cat fish *Heteropneustes fossilis* exposed to sub lethal concentrations of other toxicant was also found to increase considerably (Gill T.S., et al., 1990; Ceron, J.J et al., 1997; Kumar Hemant and Gupta A.B. 1997; Shrivastva and Singh, 1981; Saufy H., et al., 2007). The blood glucose level was increased significantly at $P < 0.01$ intoxication of metasystox after end of acute and chronic exposure periods during this toxicological investigation. It may be due to disturbing process of impulse across the neuromuscular neural junctions by inhibiting the enzymes AChE, which modulate the amount of neurotransmitter acetylcholine. The hyperglycemic conditions induced by pesticides might be explained in the part of

inhibitation of cholinesterase at neuroeffectors sites in the adrenal medulla leading to hypersecretions of adrenaline, which stimulate the break down of glycogen into glucose (Hochachka et al., 1978; Shastry K.V., and Siddiqui A. A., 1982; Ghosh T.K., 1987; Ghosh, T. K., 1989; Jyothi B., and Natrajan G. M., 1999; Kumar Hemant and Gupta A.B., 1997).

(c) Study of seasonal variation:-

The data presented in figure 43-51 showed that in acute toxicity test Hb%, TLC, ESR and MCH were lower in late summer season, while higher in beginning of rainy season in control fishes. The value of Hb% was slightly altered during summer and rainy seasons. This is because of haemodilution at the onset of rainy season. The lower activity of all parameters in the summer season may also be due to the high temperature of water. O_2 availability was also reduced in summer season. Although all the activity level were similar as shown in table 14 and 15 such as some parameters were increased and some were decreased with corresponding control fishes but the level was low during summer season and high in rainy season. It was also noticed that fish differ in their tolerance limit to temperature, dissolved oxygen (DO), pH, alkalinity, and other pollution factors. Mainly the temperature fluctuations affect the health of fishes. When water is extremely heated, much energy, oxygen and vapour is released into the air, leaving behind a high concentration of CO_2, which makes the water more acidic which increases the haemodilution. Wademeyer and Yasutake (1977) listed a number of environmental and physiological variables which show relationship to fish health. The highest blood glucose level found in rainy season in control fishes as well as the

level of glucose increase in rainy season of treated fishes indicates stimulated metabolism at low temperature and rich food supply. Several workers have also reported the various alterations in blood parameters due to different environmental stress (Charistoforides C. & Hedley-Whyte J., 1969; Bridges et al., 1976; Cech J.J., and D.E., Wohlschlag 1981; Barton B. A., and G. K. Iwama., 1991; Stein et al., 1992; Wilson R.W., Taylor E.W., 1993; Collazos et al., 1998).

Determination of LC_{50} for Metasystox on *Channa punctatus*

Table 11: First exploratory test

S.No.	Conc.ml /liter	No. of fishes	24 Hours		48 Hours		72 Hours		96 Hours	
			M	%M	M	%M	M	%M	M	%M
1	0.01	5	0	0	0	0	0	0	0	0
2	0.05	5	5	100	-	-	-	-	-	-

Table 12 : Second exploratory test

S.No.	Conc. ml/ liter	No. of fishes	24 Hours		48 Hours		72 Hours		96 Hours	
			M	%M	M	%M	M	%M	M	%M
1	0.015	5	0	0	0	0	0	0	1	20
2	0.025	5	0	0	1	20	1	40	1	60
3	0.035	5	3	60	1	80	1	100	-	-
4	0.045	5	5	100	-	-	-	-	-	-

Table 13: Definitive test

S.No.	Conc.ml /liter	No. of fishes	24 Hours		48 Hours		72 Hours		96 Hours	
			M	%M	M	%M	M	%M	M	%M
1	0.016	10	0	0	0	0	1	10	2	30
2	0.02	10	0	0	1	10	1	20	2	40
3	0.024	10	1	10	1	20	2	40	2	60
4	0.028	10	2	20	2	40	2	60	3	90
5	0.032	10	3	30	3	60	3	90	1	100
6	0.036	10	7	70	2	90	1	100	-	-
7	0.04	10	9	90	1	100	-	-	-	-

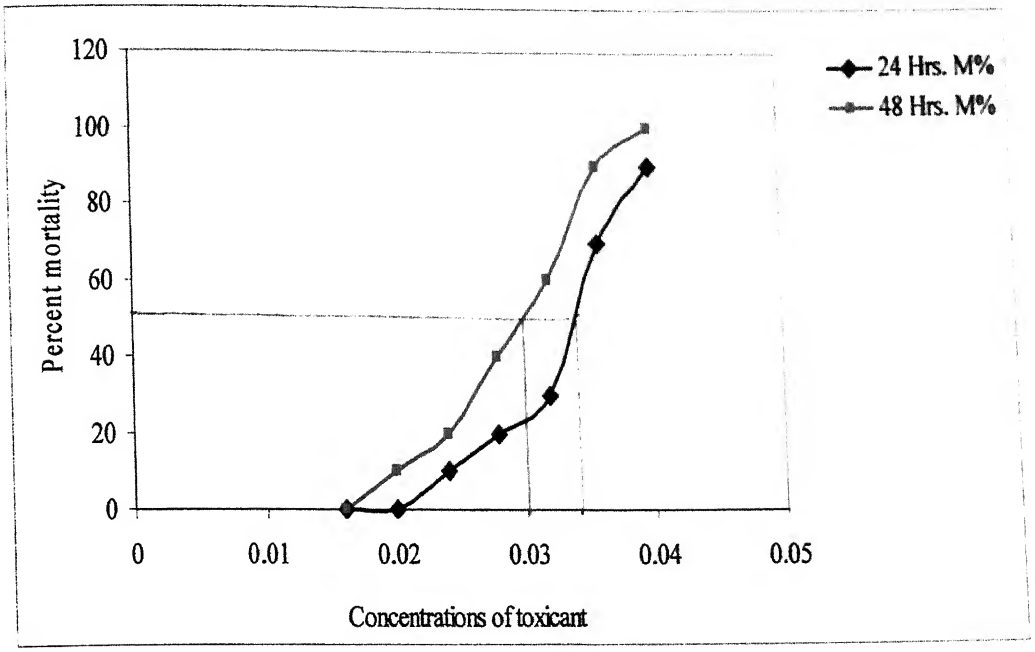


Fig 41: LC_{50} of Metasystox after 24 hours and 48

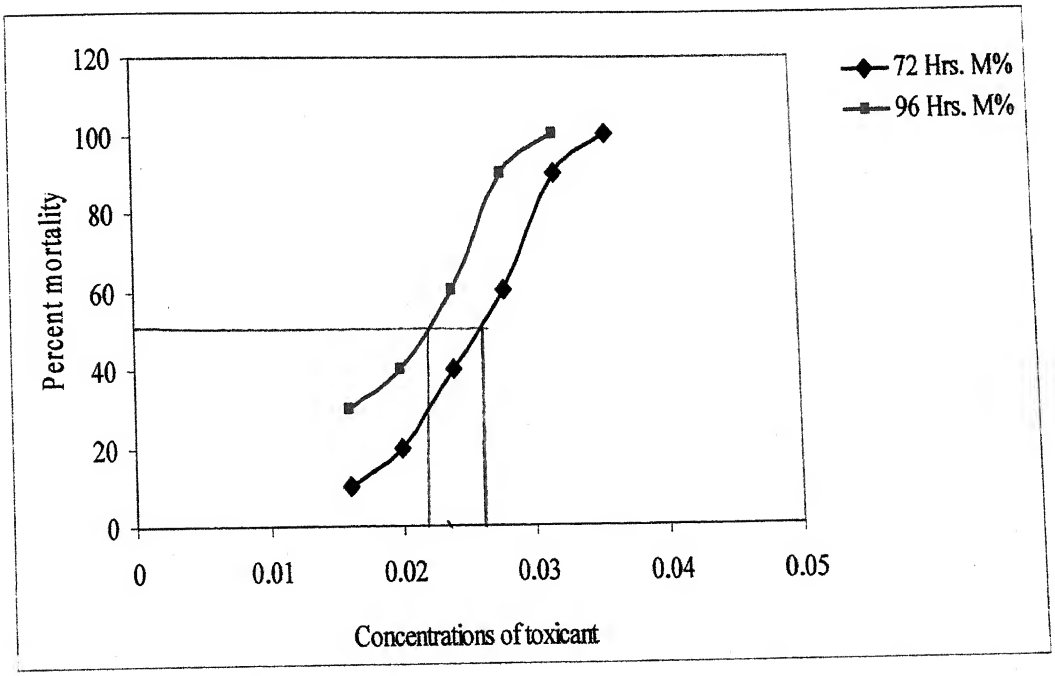


Fig 42: LC_{50} of Metasystox after 72 hours and 96 hours

Table No. 14

Effect of acute toxicity of Metasystox on selected blood parameters in fresh water fish *Channa punctatus*

S No.	Parameters	Control	Exposure Period			
			24 Hours	48 Hours	72 Hours	96 Hours
01.	Hb (g/100ml)	14.10 ±0.74	16.1* ±0.81	16.2* ±0.77	16.3* ±0.65	16.5** ±0.35
02.	TECx10^6/mm^3	3.77 ±0.10	3.87* ±0.02	3.89* ±0.05	3.91* ±0.04	3.94* ±0.02
03.	TLCx10^3/mm^3	3.3 ±0.17	2.3** ±0.11	2.4** ±0.15	2.5** ±0.15	2.7* ±0.20
04.	ESR (mm)	2.1 ±0.36	0.6** ±0..28	0.7* ±0.46	0.8* ±0.51	1.0** ±0.11
05.	PCV%	33.33 ±3.21	40.33* ±2.88	42.00* ±2.64	43.66* ±3.21	47.00** ±1.73
06.	MCH pg	37.08 ±1.01	41.50* ±2.35	41.77 * ±1.98	41.64** ±1.24	39.84* ±3.54
07.	MCHC %	44.69 ±4.62	39.96 * ±1.14	39.35* ±0.11	37.04* ±1.68	35.19* ±0.81
08.	MCV um^3	83.72 ±11.04	103.97* ±7.90	107.86* ±6.69	111.54* ±7.29	119.07** ±3.98
09.	Glucose (Units)	63.46 ±4.83	68.36* ±5.11	73.05* ±5.88	74.58* ±4.70	76.14* ±5.07

* - Significant at $P < 0.01$; ** - Significant at $P < 0.001$

Table No. 15

Effect of chronic toxicity of Metasystox on selected blood parameters in fresh water fish *Channa punctatus*

S No.	Parameters	15 Days		30 Days		45 Days	
		C	T	C	T	C	T
01.	Hb (g/100ml)	13.6 ±0.58	15.8* ±0.60	13.8 ±0.55	15.6* ±0.72	14.0 ±0.56	15.4* ±0.72
02.	TECx10^6/mm^3	3.71 ±0.11	4.01* ±0.14	3.74 ±0.12	4.03* ±0.13	3.76 ±0.13	4.06* ±0.13
03.	TLCx10^3/mm^3	3.3 ±0.40	2.4 ** ±0.37	3.6 ±0.35	2.6** ±0.35	3.4 ±0.40	2.7** ±0.10
04.	ESR (mm)	2.4 ±0.36	1.1** ±0.17	1.8 ±0.23	0.9* ±0.36	2.2 ±0.26	0.9* ±0.40
05.	PCV%	27.33 ±2.30	34.33* ±2.08	28.66 ±2.88	36.66* ±2.30	30.00 ±2.64	37.33* ±2.88
06.	MCH pg	36.82 ±0.61	39.57* ±0.93	35.86 ±2.05	39.16 * ±0.44	37.28 ±0.79	39.61* ±2.39
07.	MCHC %	46.92 ±4.00	41.14* ±2.75	48.42 ±4.78	36.57* ±1.19	40.57 ±4.84	40.56* ±2.39
08.	MCV um^3	73.91 ±5.52	89.58* ±8.45	76.52 ±6.18	92.86* ±4.46	83.30 ±2.26	97.78 ** ±1.47
09.	Glucose (Units)	61.55 ±5.01	69.93* ±5.59	64.86 ±4.99	71.35* ±5.83	65.61 ±4.11	74.85* ±4.45

* - Significant at $P < 0.01$; ** - Significant at $P < 0.001$

Graphical comparison of seasonal variation in acute toxicity experiment

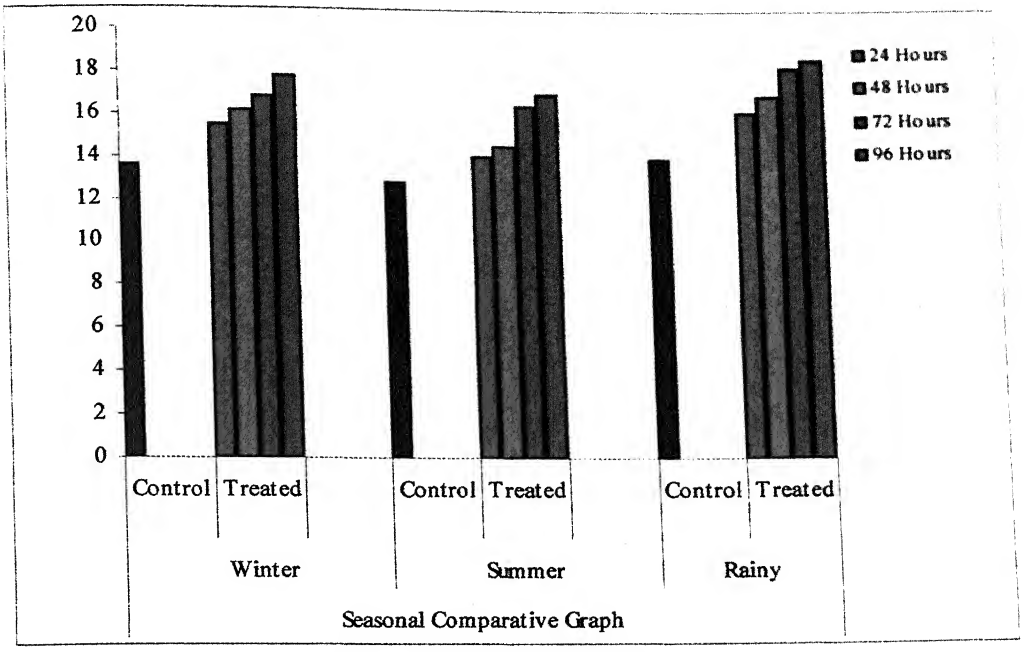


Figure 43. Haemoglobin

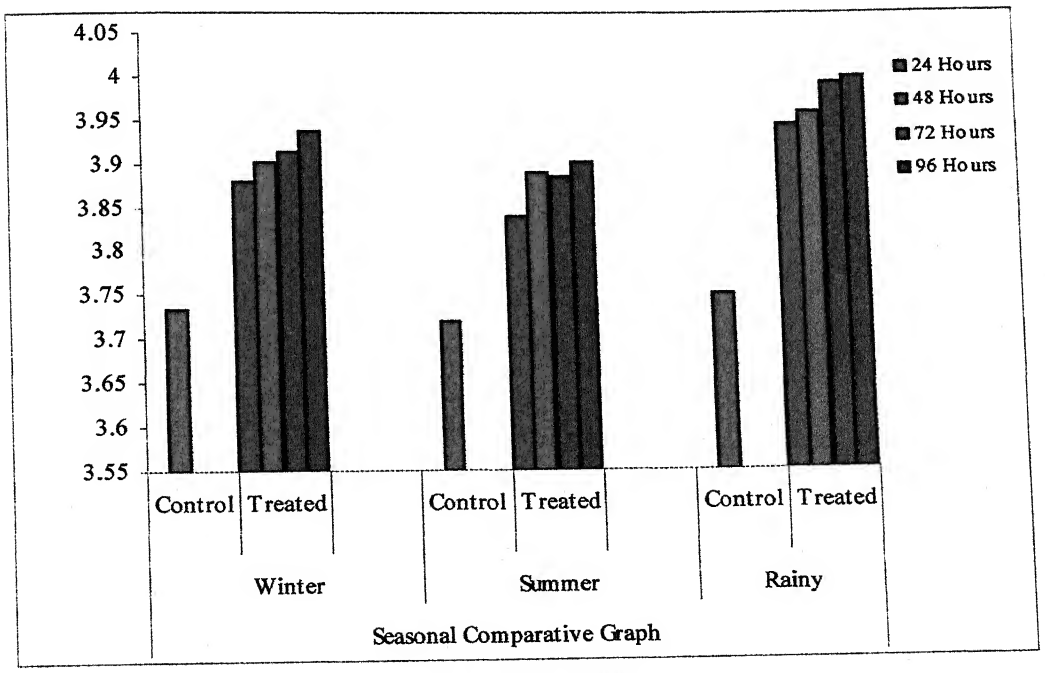


Figure 44. TEC

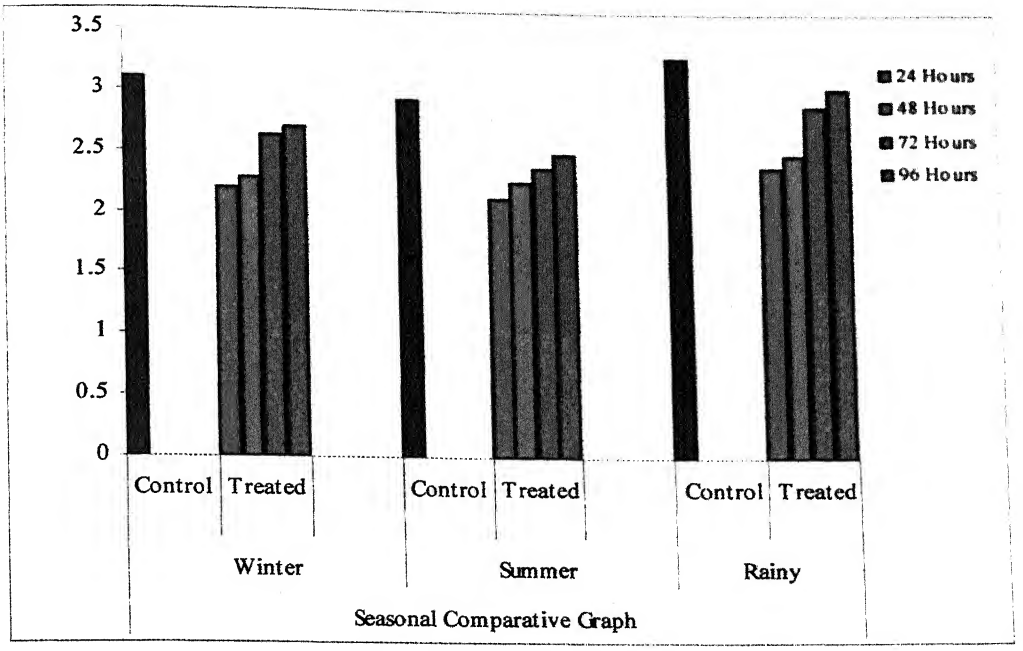


Figure 45. TLC

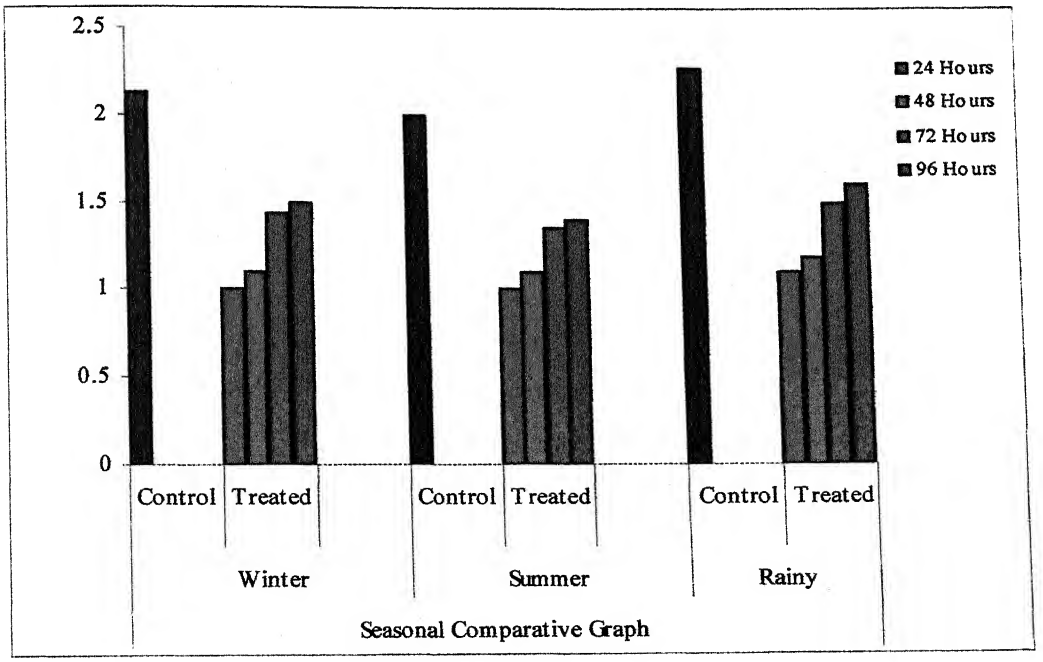


Figure 46. ESR

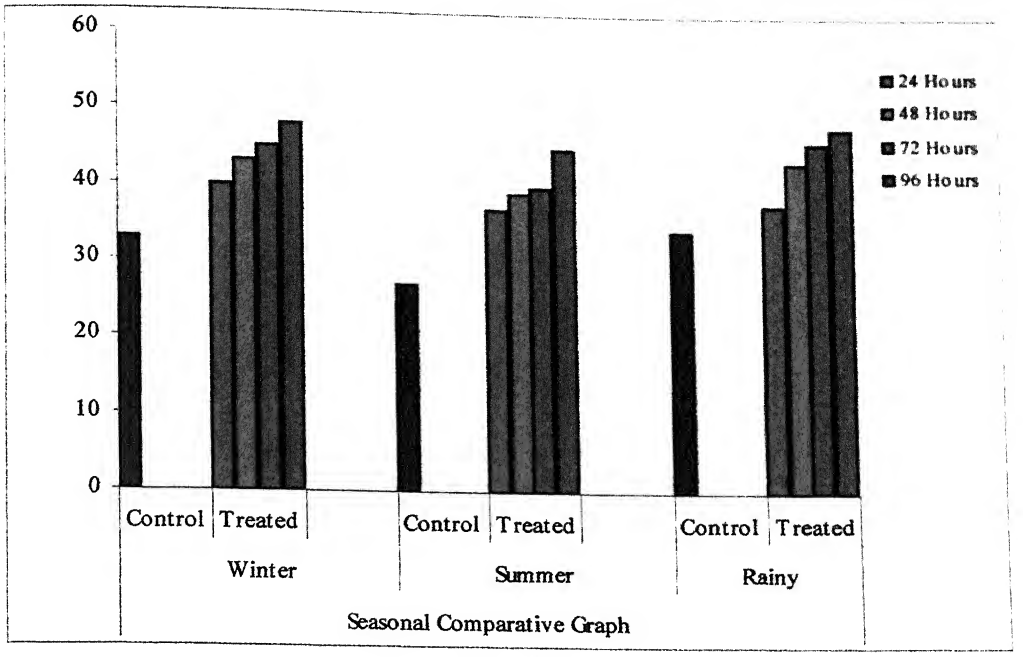


Figure 47. PCV

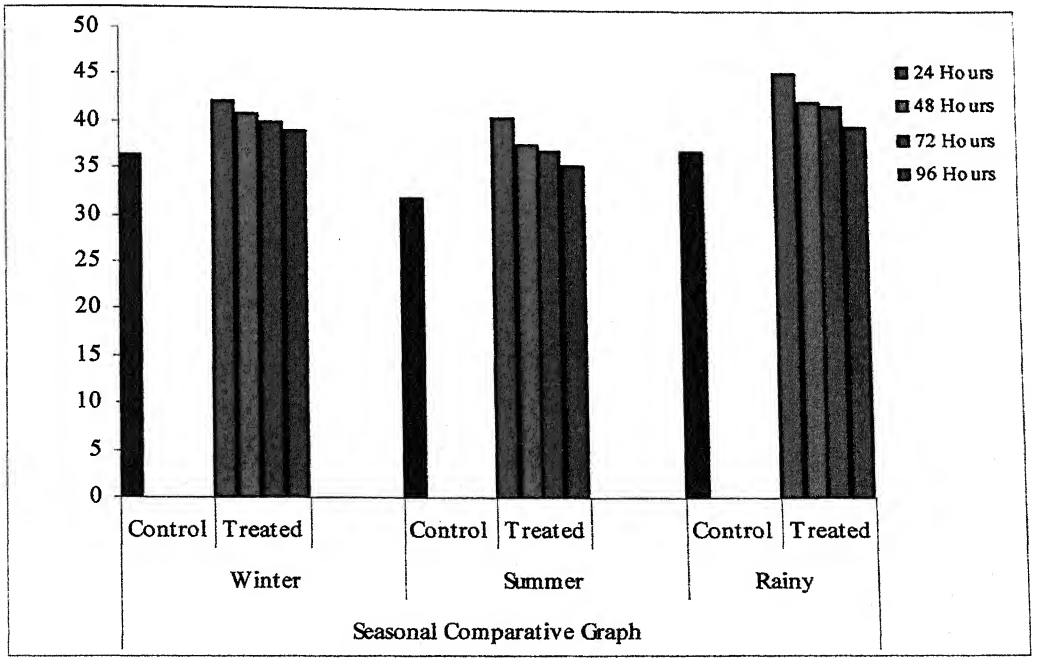


Figure 48. MCH

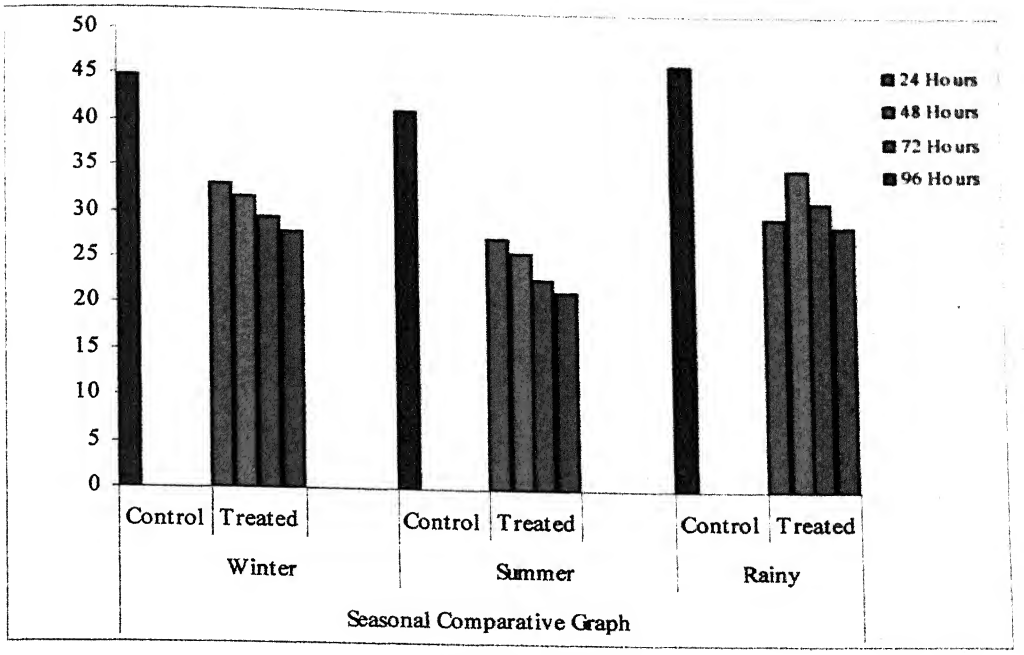


Figure 49. MCHC

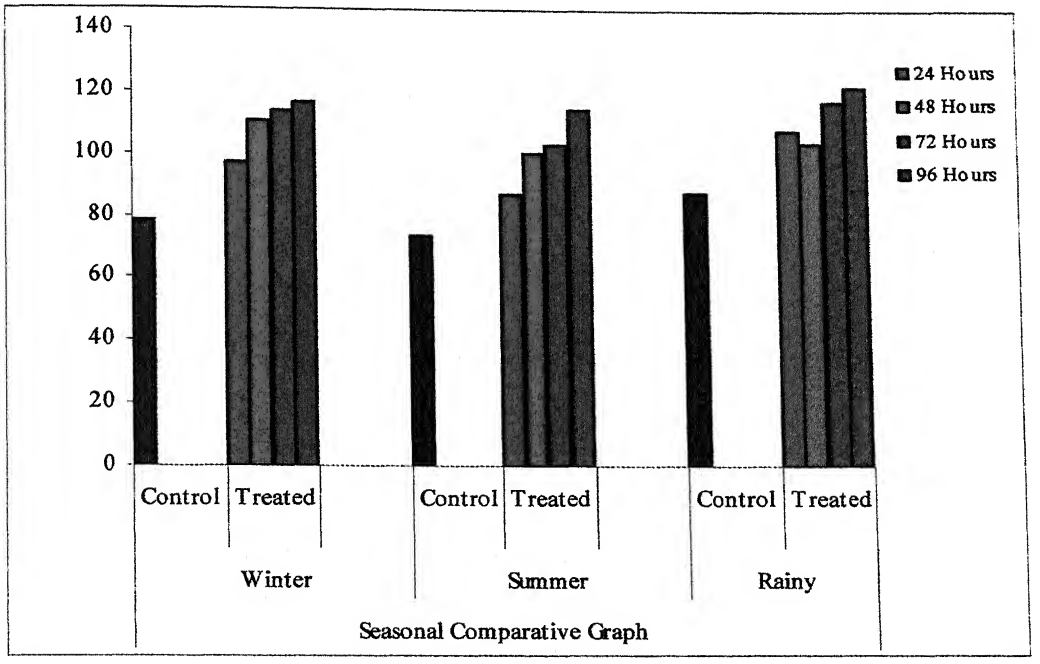


Figure 50. MCV

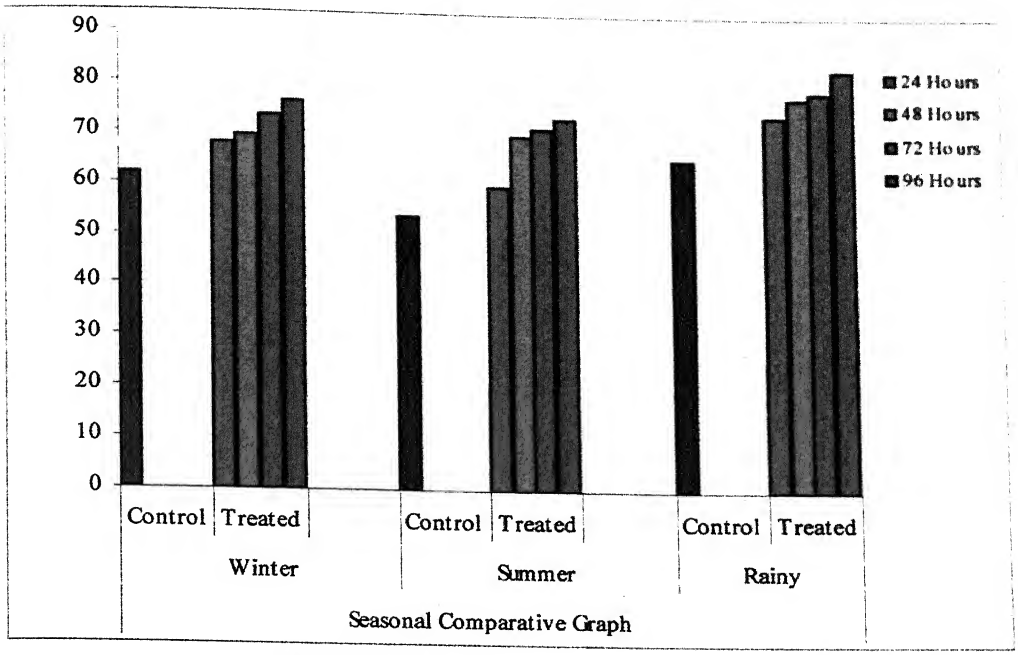


Figure 51. Blood glucose

Graphical comparison of seasonal variation in chronic toxicity experiment

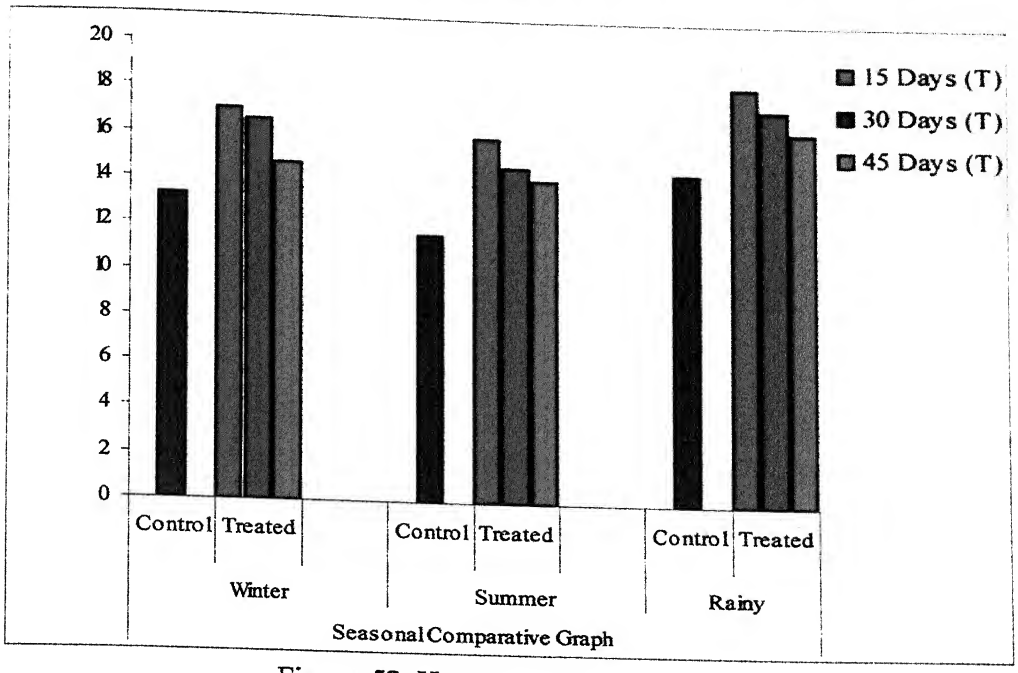


Figure 52. Haemoglobin

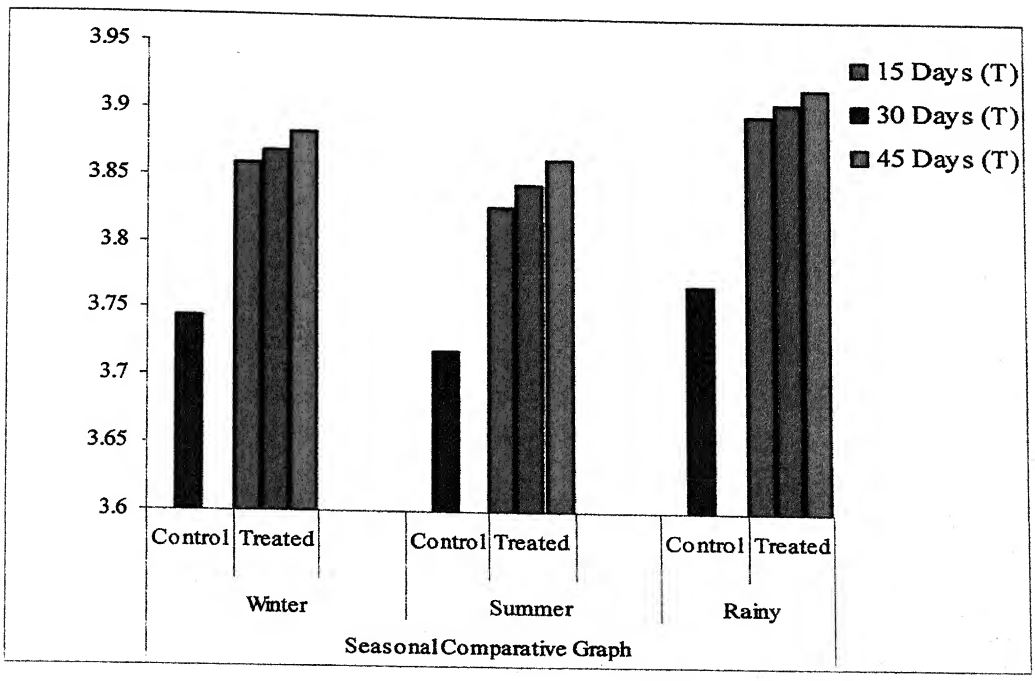


Figure 53. TEC

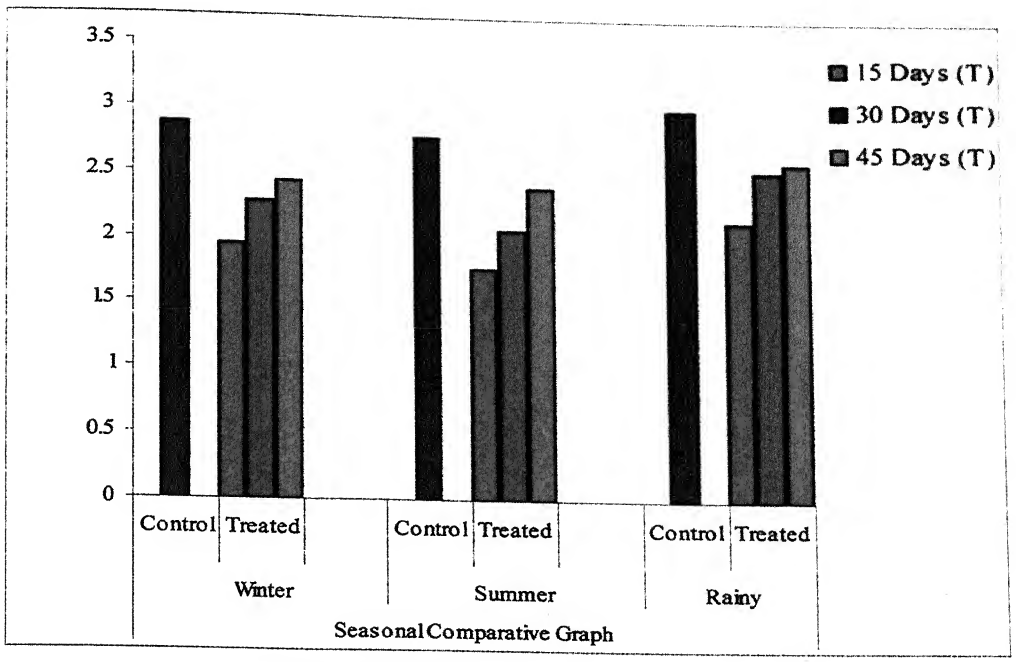


Figure 54. TLC

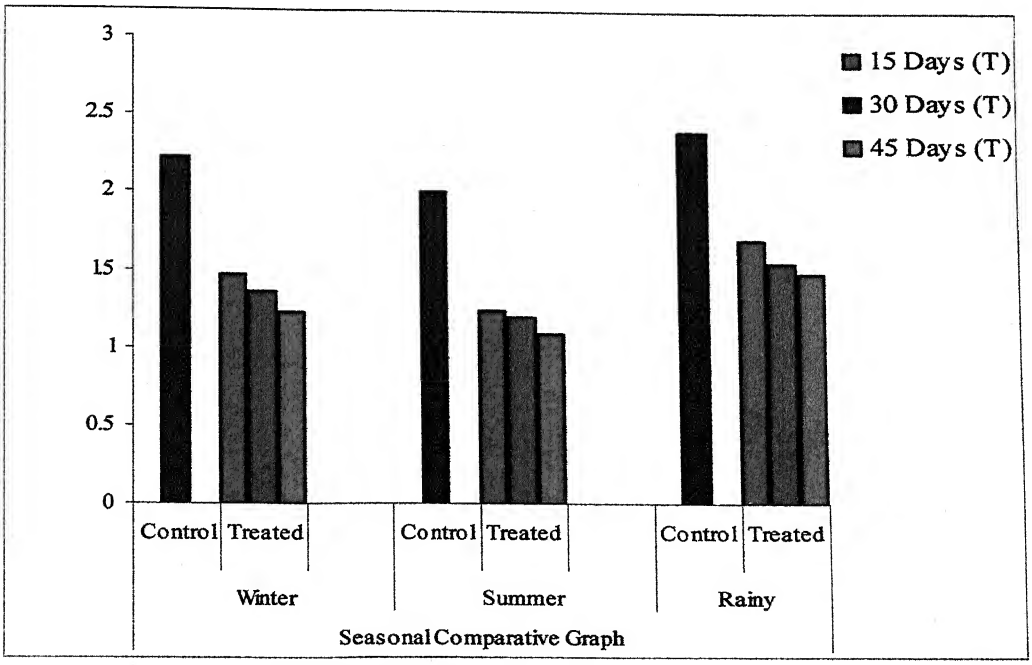


Figure 55. ESR

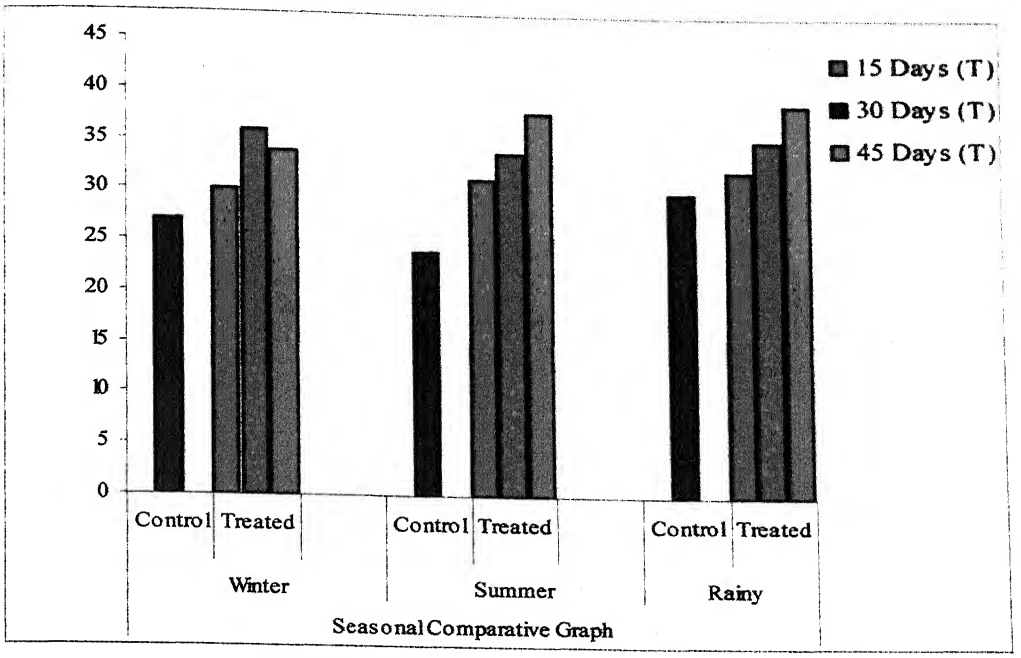


Figure 56. PCV

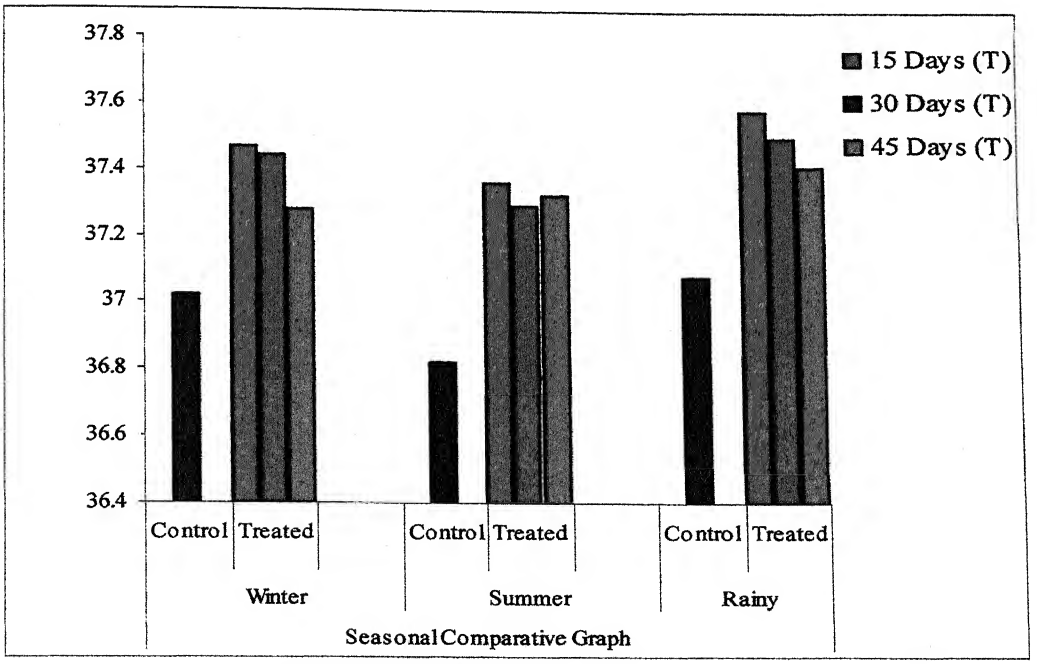


Figure 57. MCH

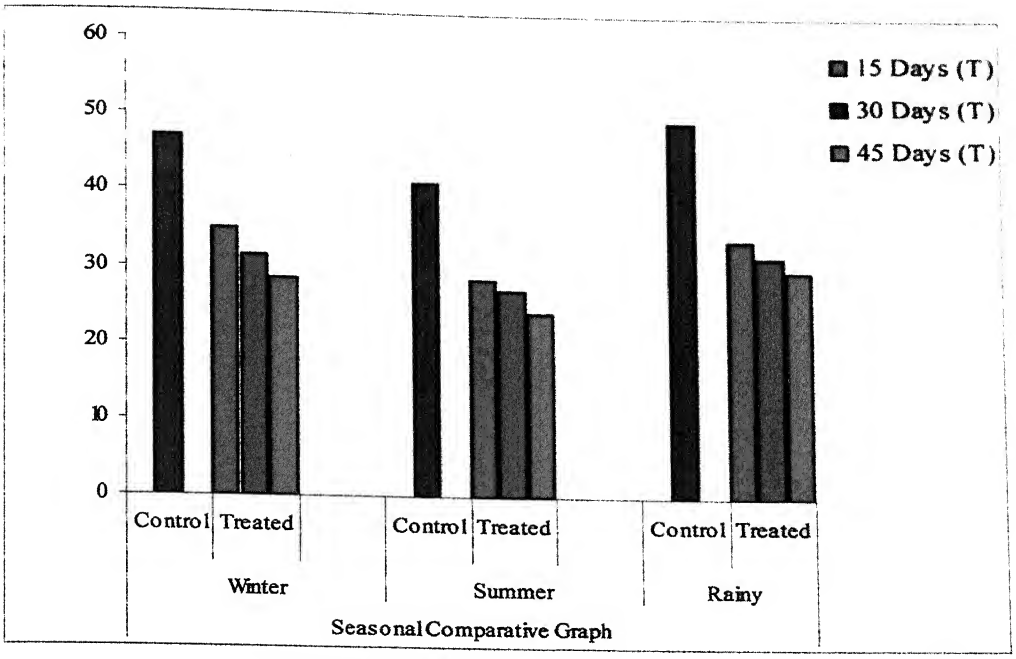


Figure 58. MCHC

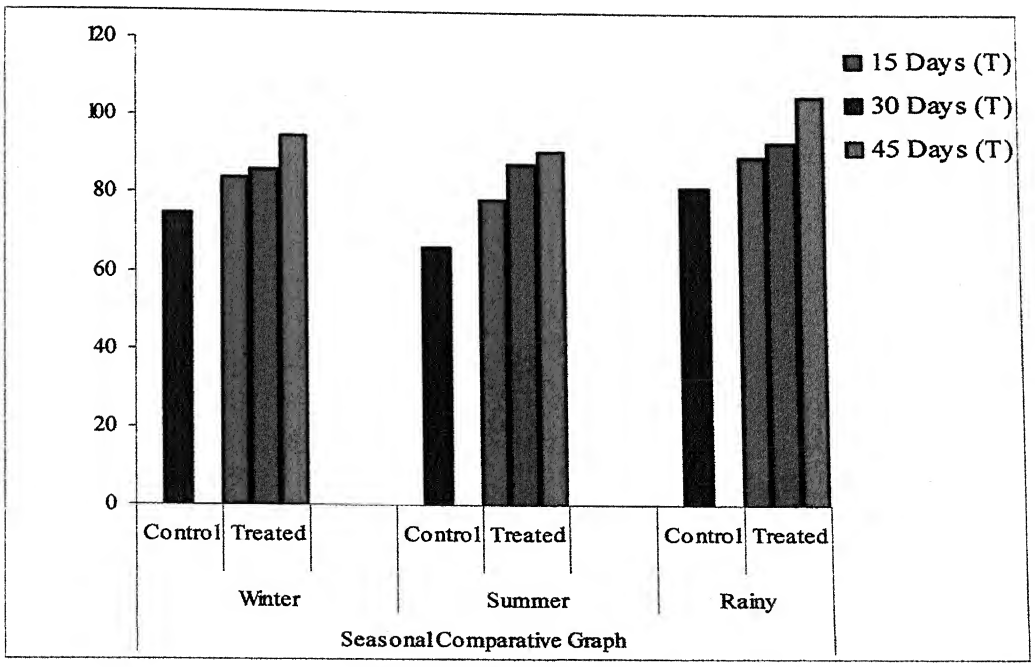


Figure 59. MCV

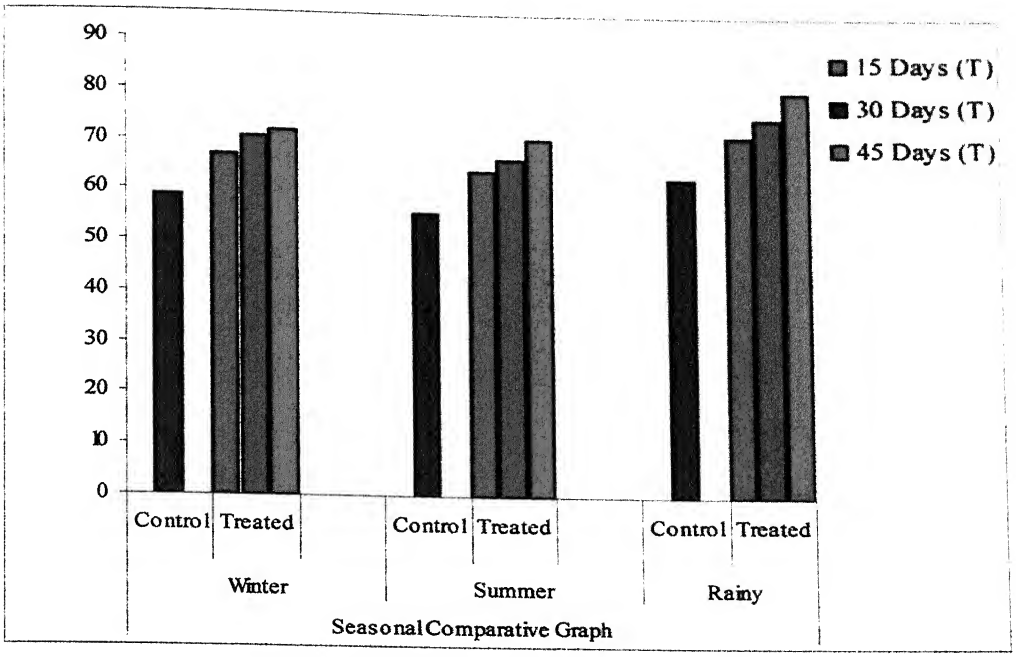


Figure 60. Blood glucose

Chapter - 8

EFFECT OF IMIDACLOPRID ON BLOOD PARAMETERS OF *CHANNA PUNCTATUS*

EFFECT OF IMIDACLOPRID ON BLOOD PARAMETERS OF *CHANNA PUNCTATUS*

Imidacloprid 17.8% S.L. (trade name Anumida) insecticide was used in this investigation. The chemical composition of this compound is (1- [6- chloro -3- pyridylmethyl]- *N*- nitroimidazolidin -2- ylideaneamine). It is manufactured by Anu products limited, Trigon road, Faridabad. India.

Results

(a) Acute toxicity bioassay:-

The LC_{50} of imidacloprid was calculated by same procedure as described earlier. In the first exploratory test 100% mortality was observed in 0.075 ml/l of imidacloprid while no mortality occurred in 0.025 ml/l. (Table 16). In second exploratory test mortality due to different concentrations were observed (Table 17). The concentrations were 0.030, 0.040, 0.050, 0.060 and 0.070 ml/liter. The 100% mortality was observed at 0.050 ml/l at 96 hours, 0.060 ml/l at 72 hours and 0.070 ml/l at 24 hours. In definitive test nine ascending series of concentrations viz 0.032, 0.036, 0.040, 0.044, 0.048, 0.052, 0.056, 0.060 and 0.064 ml/l were taken and mortality data was recorded in table 18. Then a curve was plotted between percent mortality and concentrations of imidacloprid which were used in definitive test and LC_{50} values were determined by drawing a line, intersecting through the concentration of imidacloprid at 50% mortality level (Fig 61 and 62). The LC_{50} values came to be as:-

(1.) 24 hours-0.058 ml/liter

gradually increased with increasing exposure periods. In case of ESR the value of 48 and 96 hours exposure period were also significant at $P < 0.001$. It also shows increasing trend. The levels of MCH and MCV were decreased significantly at $P < 0.01$ when compared to control fishes. While the MCHC was increased at the end of 24, 48 & 96 hours exposure periods except at 72 hours. Regarding the biochemical alterations, the glucose level increased significantly at $P < 0.01$. The level of glucose was 63.36 in untreated fishes where as fishes exposed to acute toxicity concentrations of imidacloprid showed 70.43, 71.26, 72.01, 75.19, mg/100 ml blood of glucose concentration.

In chronic toxicity bioassay (table 20) the Hb%, TEC, PCV and MCH decreased significantly ($P < 0.01$) at 15 days and 45 days after exposure of imidacloprid (1/10 of 96 hours LC_{50} concentration). The table 20 shows that the level of TEC was decreased but no significant difference were observed after the end of 15, 30 and 45 days of exposure periods. A statistically insignificant increased was observed in erythrocytes sedimentation rate expect 15 days exposure period which was significant at $P < 0.01$. TLC increased significantly during toxication. The level of MCV in treated fishes than untreated was also decreased. The level of blood glucose was increased significantly ($P < 0.01$) in treated fishes. The level of blood glucose was lower in 15 days exposure but gradually increased after the end of 30 days and 45 days exposure periods.

(c) Study of seasonal variation:-

Seasonal changes of some haematological and biochemical parameters (glucose levels) are presented in the figure 63-71 during

(2.) 48 hours-0.050 ml/liter

(3.) 72 hours-0.042 ml/liter

(4.) 96 hours-0.034 ml/liter

When the fishes were introduced into test medium with different concentrations of imidacloprid, they showed changes in their behaviour. The copious secretion of mucus by the body surface of the fishes was seen. The loss of scales, decoloration, rolling movement and loss of equilibrium in higher concentrations were observed in fishes. The fishes exhibited lethargy and erratic swimming with increment in respiratory rate. At the time of death transient hyperactivity was also observed.

(b) Haematological & biochemical study:-

The haematological and bio chemical parameters of control and Imidacloprid exposed fishes at LC_{50} concentrations are tabulated (table no. 19). The haemoglobin percentage was decreased at 24, 48, 72 and 96 hours. The difference was statistically significant at $P < 0.001$ but at 72 hours it was significant at $P < 0.01$. The haemoglobin percentage in control fishes it was 13.9 gm % where in treated fishes was 11.1, 11.4. 11.5 and 11.9 gm % for 24, 48, 72 and 96 hours of exposure periods respectively. The total erythrocytes count was also less in comparison to corresponding control. However, it was increased with increased exposure periods. Packed cell volume in control fishes was 21.66, 23.00, 23.66 and 25.33 at 24, 48, 72 and 96 hours respectively. The difference is statistically significant at $P < 0.01$. The TLC was increased significantly at $P < 0.01$. However all the values of Hb%, TEC and PCV were lower than control fishes but

acute toxicity bioassay and in figure 72-80 in chronic toxicity bioassay. The Hb%, TEC and PCV were slightly decreased during late summer season in untreated fishes. If the observation was made only in control fishes all the hematological parameters (Hb%, RBC, TEC, PCV, ESR, MCV, MCH & MCHC) and biochemical parameter (blood glucose) were decreased in summer season but slight difference was observed in winter and rainy season (fig 63-80). The haematological changes that occurred in treated fishes are similar as shown in table 19 and 20. The levels of Hb%, TEC, PCV, MCH and MCV were decreased but TLC, ESR and MCHC were increased in treated fishes when compared to untreated control during different season both in short term and long term duration as shown in fig 63-80.

Discussion

(a) Acute toxicity bioassay

The most effective insecticide against sucking insect pests currently used is imidacloprid (1- [6-chloro- 3 – pyridylmethyl] – *N* - nitroimidazolidin -2 – ylideaneamine), a neurotoxic chemical with the same mode of action as nicotine (Armbrust K.L., 2000; Matsuda et al., 2001; Tomlin 2000). It belongs to a group of insecticides referred to as the chloronicotinyl group (Kidd and James 1991; Cox et al., 1997; Tomlin 1997). It produces toxicity by binding to and over stimulating nicotinic acetylcholine (Ach) receptors on the postsynaptic membranes of neurons (Kidd and James 1991; Song et al., 1997; Tomlin 1997). Kidd and James (1991) reported LC_{50} value 211 mg/l in rainbow trout (*Oncorhynchus mykiss*) for 96 hours. George Young (2009) reported LC_{50} value of 237 mg/l in *Golden orfe*. The LC_{50}

values in invertebrates have also been reported by Sarah. J., (2008) which was 65.43 µg/l in *Chironomus tentans* for 96 hours. Imidacloprid has moderate toxicity to mammals. Tomlin (1997) reported the LD_{50} in rat (*Rattus norvegicus*) which was 460 mg/kg. Compared to the available literature on imidacloprid toxicity to fishes and other aquatic invertebrates (Liu M-Y., Casida J.E., 1993; Liu et al., 1993) our results indicate that fishes are also sensitive to imidacloprid and LC_{50} values were comparable to other workers on other animals. Very few literature are available on toxicity of imidacloprid. This study and this chemical is used as insecticide in Bundelkhand region so it become necessary to investigate the toxicity of imidacloprid. The study of imidacloprid was used for the first time in Bundelkhand region for toxicity investigation in fishes.

The behavioral changes of hyperactivity and copious secretion of mucus by the body surface of the fishes and other effects like as loss of scales, decoloration, rolling movement and loss of equilibrium in higher concentrations were observed in this study. This may be due to intoxication of imidacloprid on the central nervous system. Increased opercular movement of fishes as soon as introduced in toxic media involves considerable energy spending and there by create a greater require of oxygen. Similar changes in behavior of fresh water fishes due to exposure of other pesticides were reported by other biologists using other pesticides (Mukhopadhyay, P.K and Dehadrai, P.V. 1984; Olla et al., 1980; Salim Mustfa and Ajmal Murad 1984; Lal et al., 1984; Naqvi and Howkins 1988; Gray, R.H. 1990; Little et al., 1993b; Baldwin et al., 1994; Doving K. V., 1995; Kumar Hemant and A.B. Gupta 1997; Beauvais et al., 2000 ; Brewer, S.K., et al., 2001;

Sadhu et al., 2001; Vatukuru S.S., 2005).

(b) Haematological and biochemical study:-

In 1992, Imidacloprid was introduced as a new insecticides. It is most active against sucking insects so it is widely used in agricultural programms. In India, renewal of agricultural programme has increased utilization of pesticides some of which are known to be highly toxic to non–target organisms such as fishes (Shrivastva, A. K. and N. N. Shrivastva. 1987; Benerjee G. and Rajendranath 1990; Benerji, G. and Rajendranath 1988; Mohapatra, B.C. and Nobal. 1992; Sastry, A. and R. A. Agrawal. 1993; Tiwari, C., 1995; Watson et al., 1997; Kumar et al., 1999; Das, R., 2000; Alam, M. N. and D. N. Sadhu, 2001). However, this positive trend is helpful in increasing food production and improving crop health the use of pesticides has negative ramifications for fish and other aquatic life inhabiting ponds, lakes, rivers, streams. On reaching aquatic ecosystems, such chemicals can cause serious harm to fishes and other aquatic organism. Studies have shown that when the water quality is affected by toxicants, physiological changes will be reflected in the values of one or more of the haematological parameters (Van Vuren, 1986; Shrivastva, A. K. and N. N. Shrivastva. 1987; Benerji, G., and Rajendranath 1988; Barto B. A., and G. K., Iwama. 1991; Fernando M.D., and E.A., Moliner 1991; Mohapatra B.C., and Nobal. 1992;).

For this approach many Bioresearches used haematological and biochemical parameters as biomarkers. In fishes, exposure to Imidacloprid pollutants can induce either increases or decreases in haematological levels. Many workers have been selected

heamatological and biochemical parameters to determine the effected biological activities on test organism because they accept it, that the blood parameters are the best biomarkers to investigate the hazardous effect of pollution caused by various toxicants (Verma et al., 1981; Mishra et al., 1989; Sarkar 1990a; Sarkar 1990b; Sreenivasulu et al., 1991; Sadhu D.N., 1993; Wilson R.W.; Taylor E.W., 1993; Sastry A. and R. A. Agrawal. 1993; Jain R. and K.D., Mishra. 1995; Tiwari C., 1995; Kumar et al., 1997; Watson W.J., et al., 1997; Kumar S., et al., 1999; Das R., 2000; Delor et al., 2000; Alam M. N. and D. N., Sadhu 2001; Mishra et al., 2001; Singh S. and D. N., Sadhu 2001).

The Hb%, RBC, PCV%, the oxygen carrying capacity of blood significantly decreased after both acute and chronic exposure periods to imidacloprid compared to corresponding control. This may be due to the impairment oxygen supply to various tissues, thus resulting a slow metabolic rate and low energy production (Ahmad F., et al., 1995). Similar response were observed in the report of Blaxhall (1972); Wedemeyer et al., (1976); Gill and Pant (1985); Chakraporti P., (1986); Hoemechaudhuri (1986); Mishra B. K., (1993); Ravindra Nath and Benerjee V., (1999). The significant decreased in total RBC count, PCV, and Hb % also might be due to hypo stimulation to erythropoietic tissue (Lone et al., 1976). Decrease in TEC, Hb% and PCV% were also presented in the work of Mahajan and Juneja 1979; Mishra B. K., 1993 in *Channa punctatus* and *Heterpneustes fossilis*.

The alterations in haematological parameters were brought about by Imidacloprid. The decreased in Hb% was seen in the present study causing anaemia which is due to decrease synthesis of red blood cells (Morgen et al., 2000; and Nuri et al., 2003). The response of

MCH and MCV were decreased while the value of MCHC was fluctuated in acute exposure but in chronic exposure it was increased slightly. Similar response were also observed by (Janardhana Reddy et al., 1998; Verma G.P., and Pranamita Panighari 1998; Vatukaru S.S., 2005).

Increase in the TLC level also exhibited stress condition. The increase TLC can be correlated with an increase in antibodies production which helps in survival and recovery of the fishes exposed to imidacloprid (Jhosi P., Deep H., 2002; Ramesh M., and Saravanan M., 2008). An increased in TLC and ESR have negative correlation with TEC (Kumar B., and Benerjee V., 1990; Goel K A., and Maya 1986). Malla F.A.G., Sharma (2009) and Singh S., and Bhati DPS., (1991) also reported increased values of ESR were found in *Channa punctatus* intoxication of Chlorpyrifos (Chaturvedi L. D. and Agrawal K., 1993; Geol K.A., and Maya 1986; Kumar B., and Banerji V. 1990; Singh S., and Bhati DPS., 1991).

The blood glucose level was increased in treated fishes exposed to imidacloprid after both acute and chronic exposure. It was noticed that hyperglycemic condition arise in fresh water fishes following exposure to pesticides (Shrivastava and Singh 1987; Kumar Hemant and A.B., Gupta 1997). The increase in circulating glucose level is also correlated with increased concentration of the circulating acetylcholine under stressed conditions. In this study increased blood glucose might have resulted from gluconeogenesis to provide energy for the increased metabolic demands imposed by imidacloprid stress. Many other bio researchers have also been reported the hypoglycemic condition of fish intoxication of various organophosphorus pesticides

(Hochachka, P.W., 1978; Shaikh, Y.A., and P.K., Hiradher 1985; Ghosh, T. K., 1989; Shobha et al., 1989; Chandrasekhar and Jayabalan S. 1993; Gill T.S., et al., 1990 Sikoki, F. D., & Enajekpo, H.O.S. 1991; Ghosh R, Shrotri R .V., 1992; Ceron J.J., et al., 1997; Kumar Hemant and Gupta A.B., 1997).

(c) Study of Seasonal variation:-

The data obtained in figure 63-71 showed that Haemoglobin percentage, TEC, PCV, MCH and MCV decreased but TLC, ESR, MCHC and blood glucose level were increased following exposure to imidacloprid from control fishes both in acute and chronic experiment. These changes were presented in table 19 and 20, but the concentrations of above parameters in control and treated fishes were lowest in summer and then increased at the end of late summer. The increased PCV in rainy season was observed in control fishes. The changes in haematological and biological parameters may be due to low value of dissolve oxygen in summer. Hence low oxygen availability from water increases the process of haemodilution in summer followed by haemoconcentration in winter. The another reason of low blood parametric value in late summer was increasing temperature , increasing CO_2, and decreasing the oxygen affinity in the blood (Eddy F.B. 1973). The high level of CO_2 causes low ph value (chapter 4) in summer is the indication of haemodilution. The temperature of water had a direct influence on the toxicity of many pollutants on the growth of fishes. Therefore the blood parameters were low in summer season. (Akira Kakuno and Jiro Koyama 1994; Gross et al., 1996; Best et al, 2001).

Determination of LC_{50} for Imidacloprid on *Channa punctatus*

Table 16: First exploratory test

S.No.	Conc.ml /liter	No. of fishes	24 Hours		48 Hours		72 Hours		96 Hours	
			M	%M	M	%M	M	%M	M	%M
1	0.025	5	0	0	0	0	0	0	0	0
2	0.075	5	5	100	-	-	-	-	-	-

Table 17: Second exploratory test

S.No.	Conc.ml/liter	No. of fishes	24 Hours		48 Hours		72 Hours		96 Hours	
			M	%M	M	%M	M	%M	M	%M
1	0.03	5	0	0	0	0	0	0	1	20
2	0.04	5	0	0	1	20	1	40	2	80
3	0.05	5	1	20	1	40	2	80	1	100
4	0.06	5	3	60	1	80	1	100	-	-
5	0.07	5	5	100	-	-	-	-	-	-

Table 18 : Definitive test

S.No.	Conc.ml/liter	No. of fishes	24 Hours		48 Hours		72 Hours		96 Hours	
			M	%M	M	%M	M	%M	M	%M
1	0.032	10	0	0	1	10	1	20	2	40
2	0.036	10	0	0	1	10	2	30	3	60
3	0.04	10	0	0	2	20	2	40	4	80
4	0.044	10	1	10	2	30	3	60	3	90
5	0.048	10	2	20	2	40	3	70	3	100
6	0.052	10	3	30	3	60	3	90	1	100
7	0.056	10	4	40	3	70	3	100	-	-
8	0.06	10	6	60	2	80	2	100	-	-
9	0.064	10	8	80	2	100	-	-	-	-

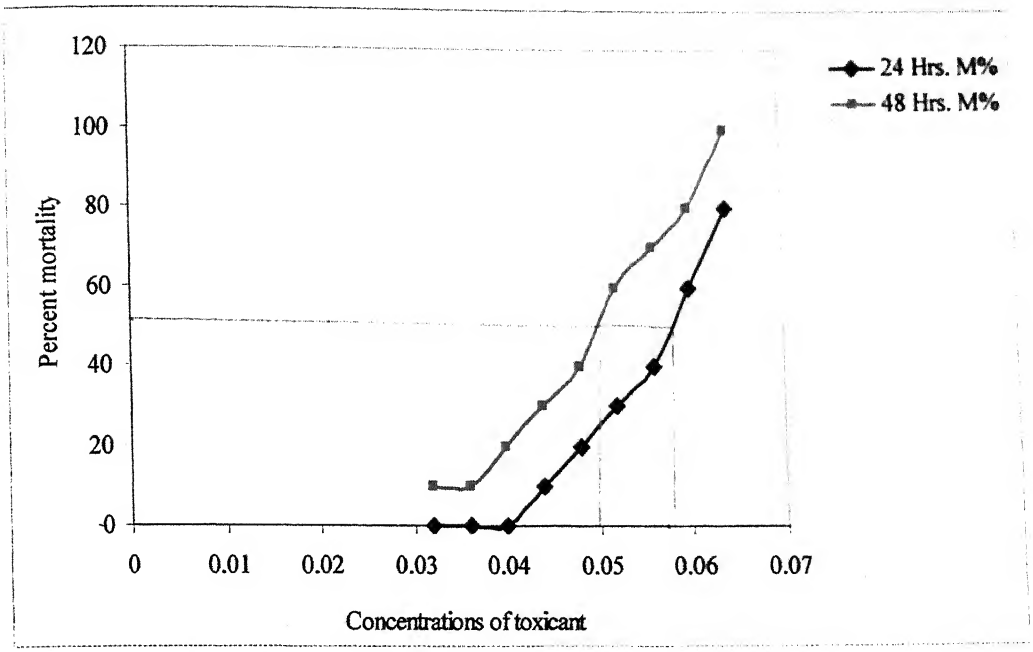


Fig 61: LC_{50} of Imidacloprid after 24 hours and 48 hours

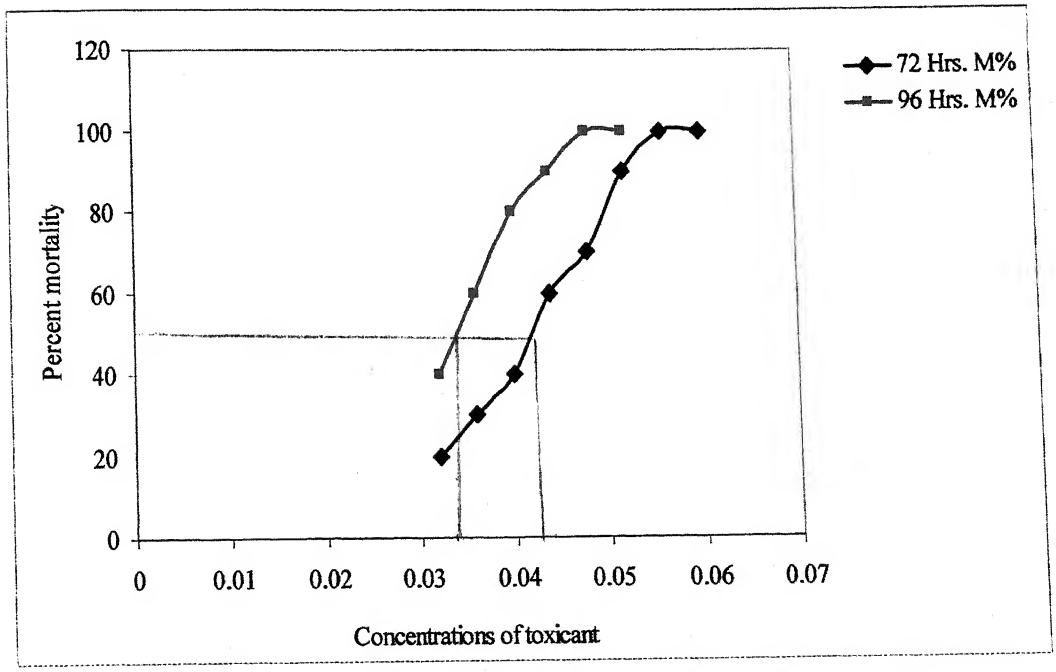


Fig 62: LC_{50} of Imidacloprid after 72 hours and 96 hours

Table No. 19

Effect of acute toxicity of Imidacloprid on selected blood parameters in fresh water fish *Channa punctatus*

S No.	Parameters	Control	Exposure Period			
			24 Hours	48 Hours	72 Hours	96 Hours
01.	Hb (g/100ml)	13.9 ±0.60	11.1** ±0.75	11.4** ±0.35	11.5* ±1.00	11.9** ±0.20
02.	TECx10^6/mm^3	3.77 ±0.11	3.46* ±0.05	3.48* ±0.07	3.50* ±0.06	3.52* ±0.50
03.	TLCx10^3/mm^3	3.1 ±0.40	3.6* ±0.58	4.0* ±0.41	4.1* ±0.43	4.2* ±0.70
04.	ESR (mm)	1.9 ±0.11	3.8* ±0.28	3.6** ±0.57	3.8* ±0.28	4.0** ±0.64
05.	PCV%	30.00 ±3.60	21.66* ±1.52	23.00* ±3.48	23.66* ±2.08	25.33* ±2.51
06.	MCH pg	36.85 ±0.47	32.26* ±2.63	33.09 * ±1.66	33.67* ±2.11	34.10* ±1.59
07.	MCHC %	46.63 ±2.42	52.92 * ±2.35	51.94* ±6.59	46.05* ±2.35	42.83* ±0.42
08.	MCV um^3	79.43 ±7.26	62.54* ±3.99	67.96* ±5.94	67.56* ±6.29	72.11* ±6.17
09.	Glucose (Units)	63.36 ±6.29	70.43* ±5.24	71.26* ±3.48	72.01* ±6.75	75.19* ±7.05

* - Significant at $P < 0.01$; ** - Significant at $P < 0.001$

Table No. 20

Effect of chronic toxicity of Imidacloprid on selected blood parameters in fresh water fish *Channa punctatus*

S No.	Parameters	15 Days		30 Days		45 Days	
		C	T	C	T	C	T
01.	Hb (g/100ml)	13.9 ±0.60	11.5** ±0.30	14.1 ±0.55	11.8** ±0.65	14.2 ±0.56	12.1** ±0.34
02.	TECx10^6/mm^3	3.77 ±0.11	3.17 ±0.20	3.82 ±0.09	3.24 ±0.05	3.78 ±0.25	3.31* ±0.05
03.	TLCx10^3/mm^3	3.1 ±0.40	4.0 * ±0.23	3.3 ±0.30	4.3* ±0.34	3.4 ±0.20	4.3* ±0.79
04.	ESR (mm)	1.9 ±0.11	3.3** ±0.28	2.0 ±0.05	3.9 ±0.36	2.1 ±0.28	4.1 ±0.23
05.	PCV%	27.66 ±2.88	20.00* ±2.48	29.66 ±3.05	21.00* ±3.48	32.33 ±2.30	23.00* ±3.46
06.	MCH pg	36.85 ±0.47	36.34* ±1.12	36.89 ±0.52	36.58 * ±1.95	37.69 ±1.10	36.47* ±0.98
07.	MCHC %	50.59 ±5.47	55.71* ±6.53	46.55 ±3.52	57.19* ±6.49	45.47 ±3.75	53.27* ±6.84
08.	MCV um^3	73.41 ±7.73	63.07* ±11.38	77.70 ±8.24	64.73* ±10.55	85.71 ±6.82	69.33 * ±10.37
09.	Glucose (Units)	59.40 ±7.58	67.82* ±8.41	63.29 ±7.84	74.17* ±8.74	64.98 ±4.67	76.23* ±7.05

* - Significant at $P < 0.01$; ** - Significant at $P < 0.001$

Graphical comparison of seasonal variation in acute toxicity experiment

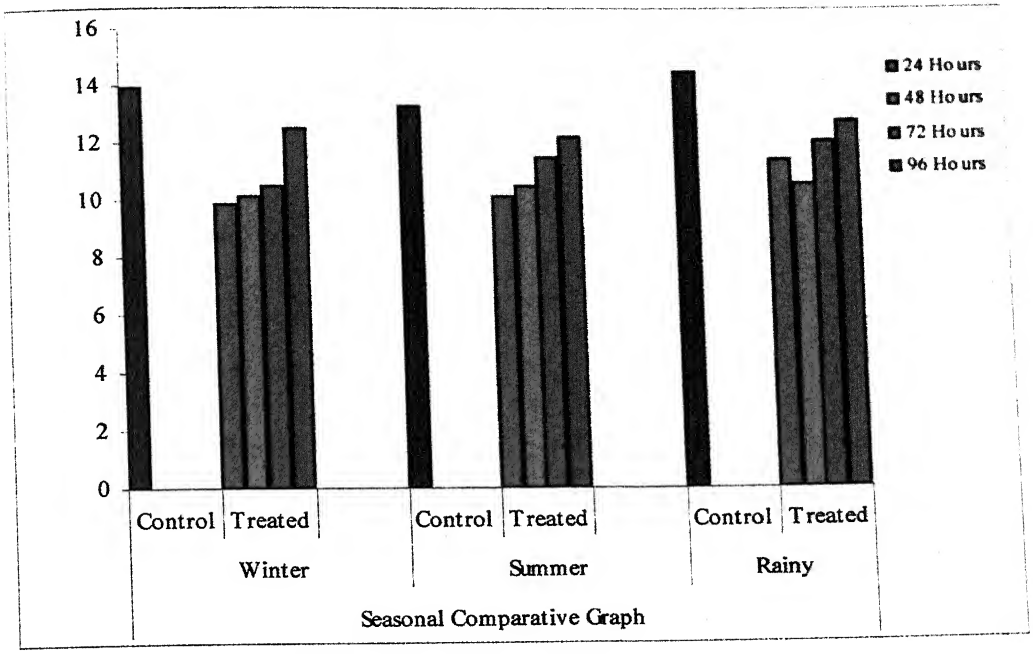


Figure 63. Haemoglobin

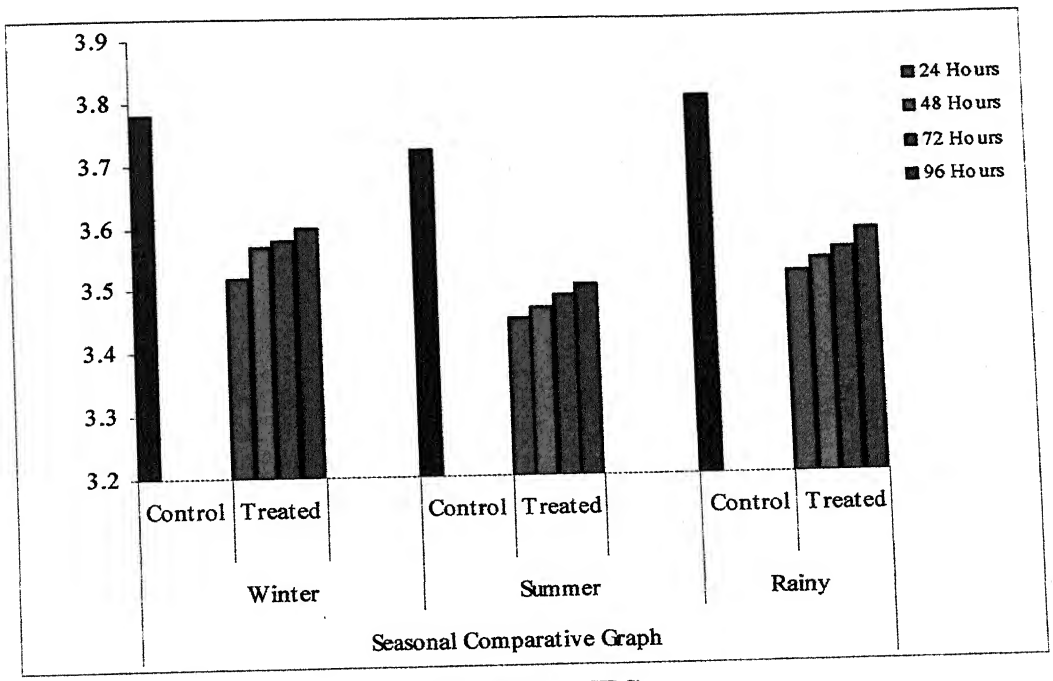


Figure 64. TEC

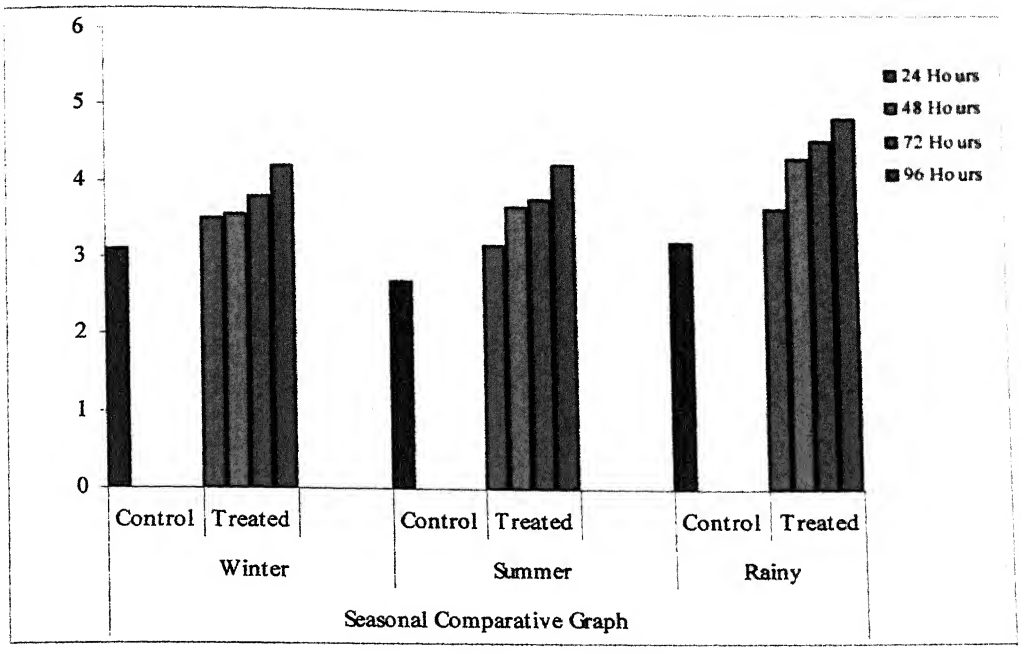


Figure 65. TLC

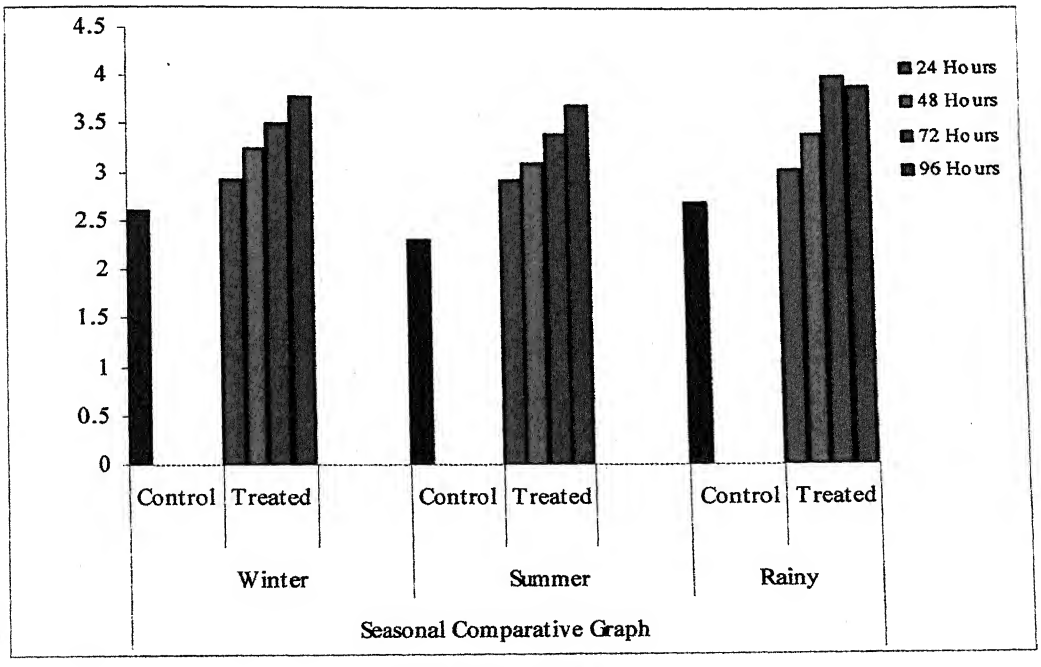


Figure 66. ESR

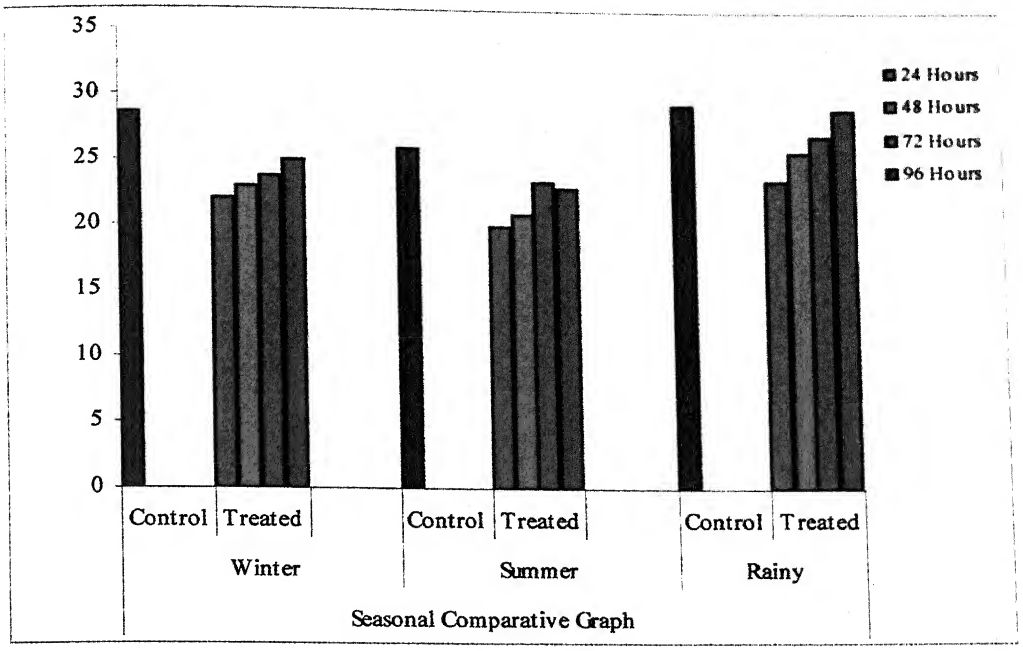


Figure 67. PCV

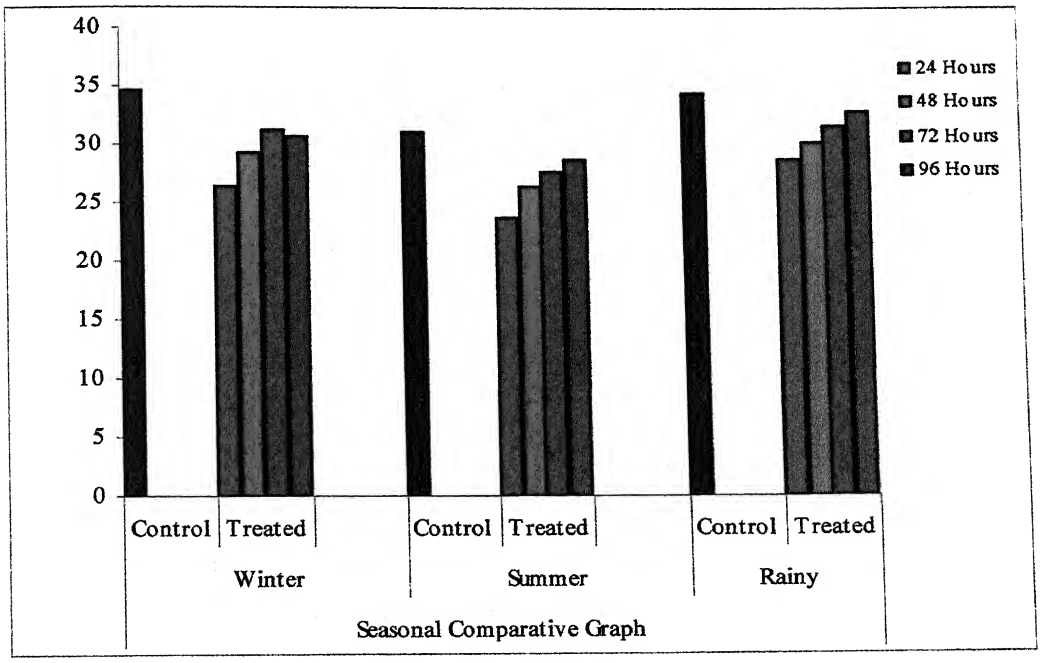


Figure 68. MCH

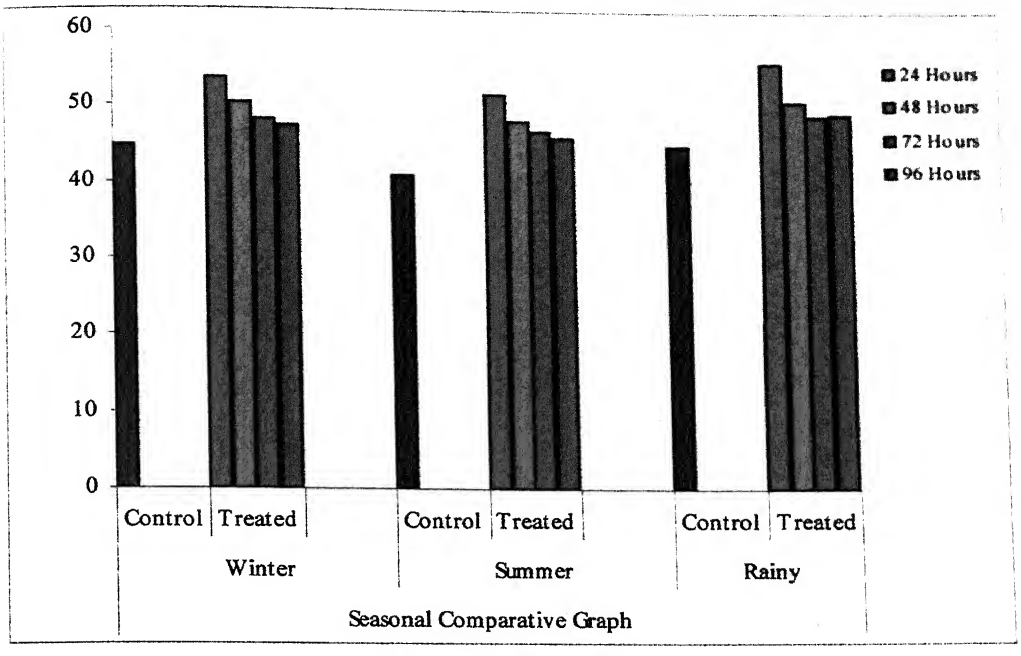


Figure 69. MCHC

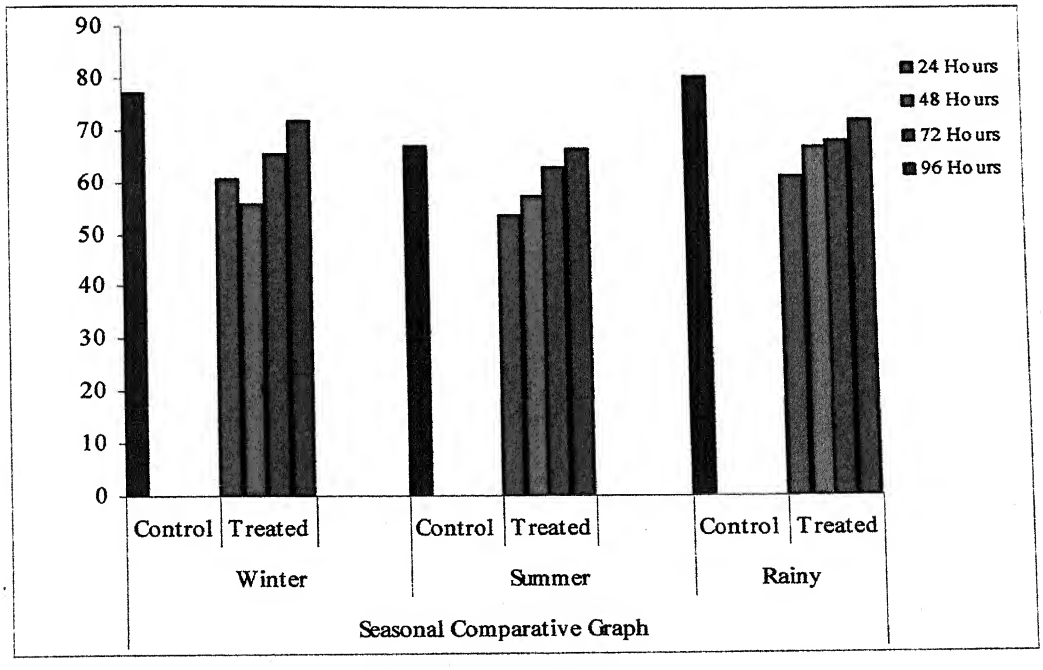


Figure 70. MCV

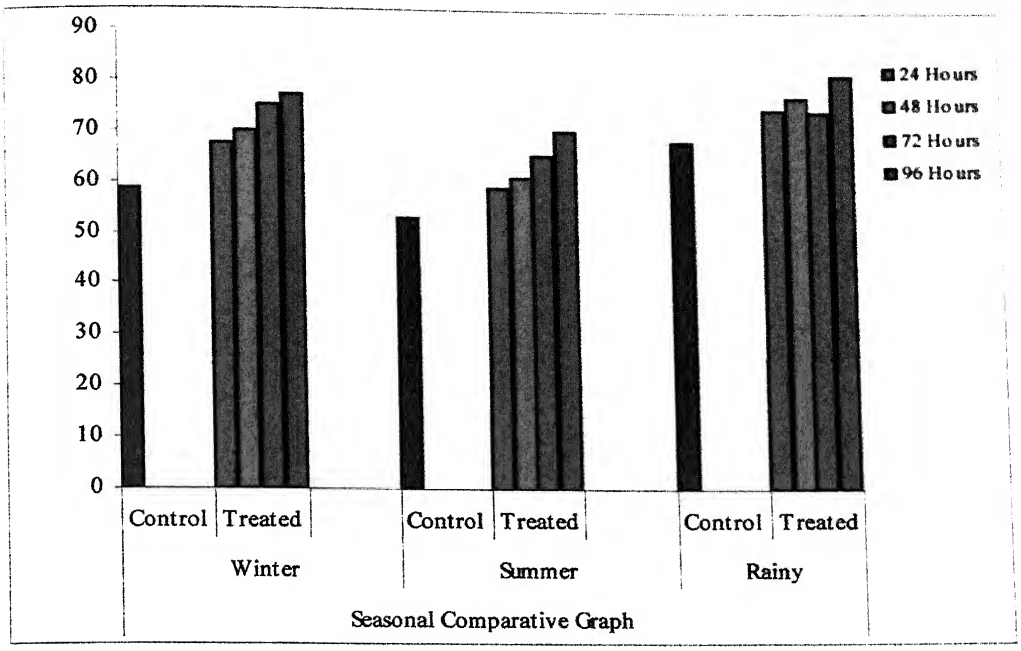


Figure 71. Blood Glucose

Graphical comparison of seasonal variation in chronic toxicity experiment

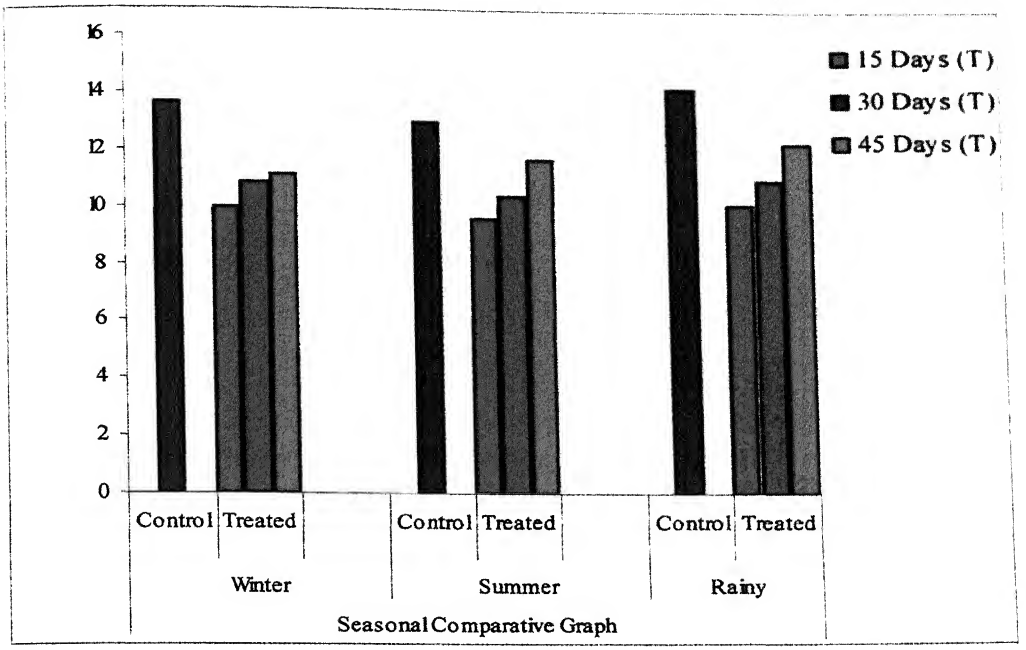


Figure 72. Haemoglobin

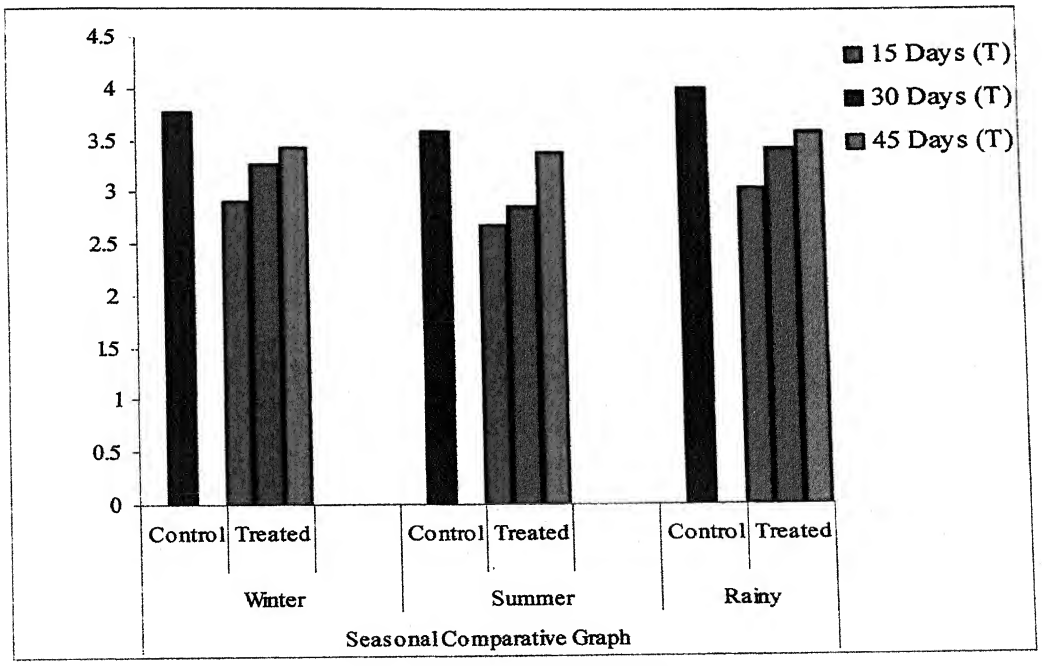


Figure 73. TEC

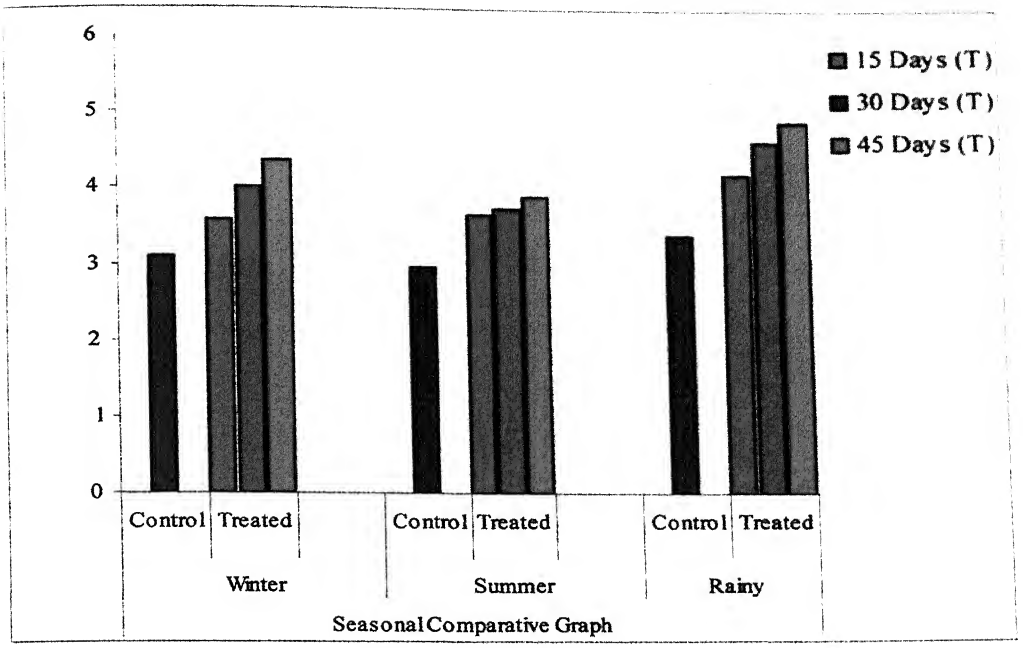


Figure 74. TLC

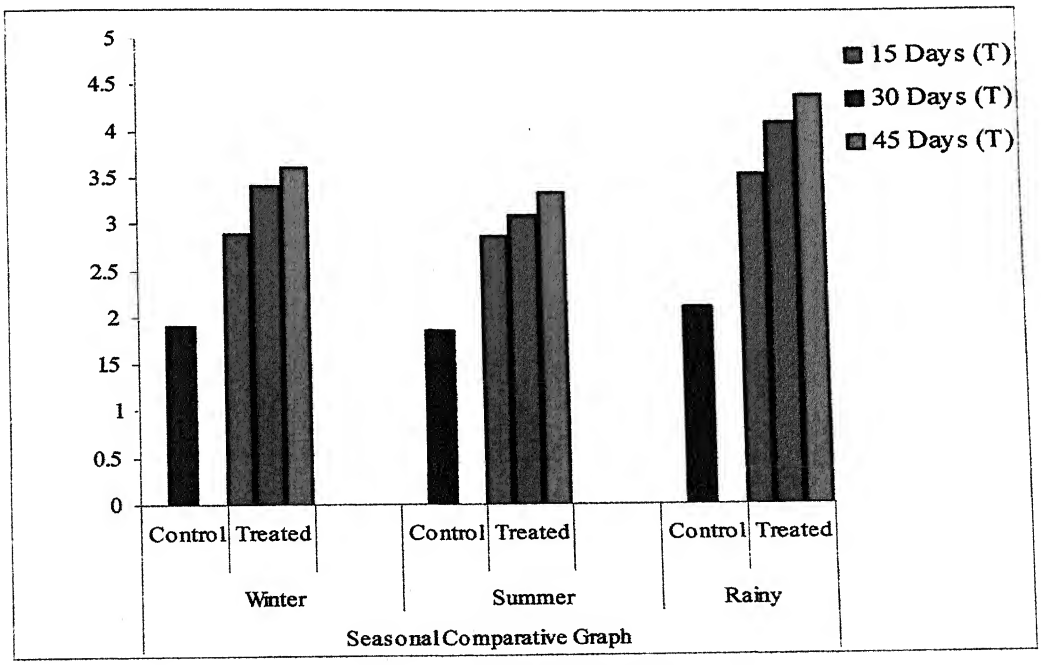


Figure 75. ESR

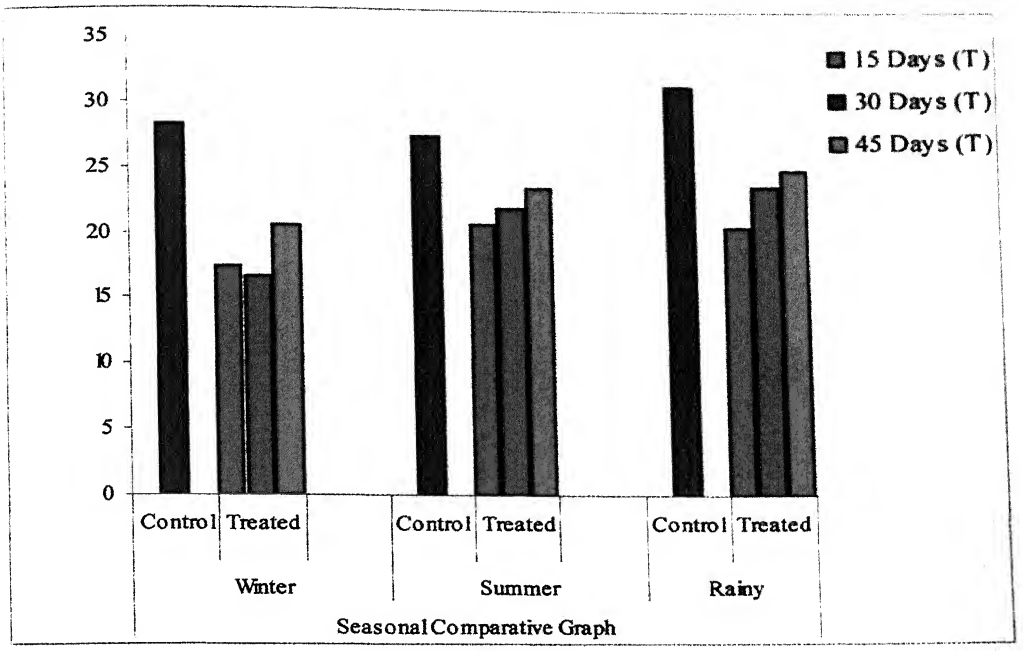


Figure 76. PCV

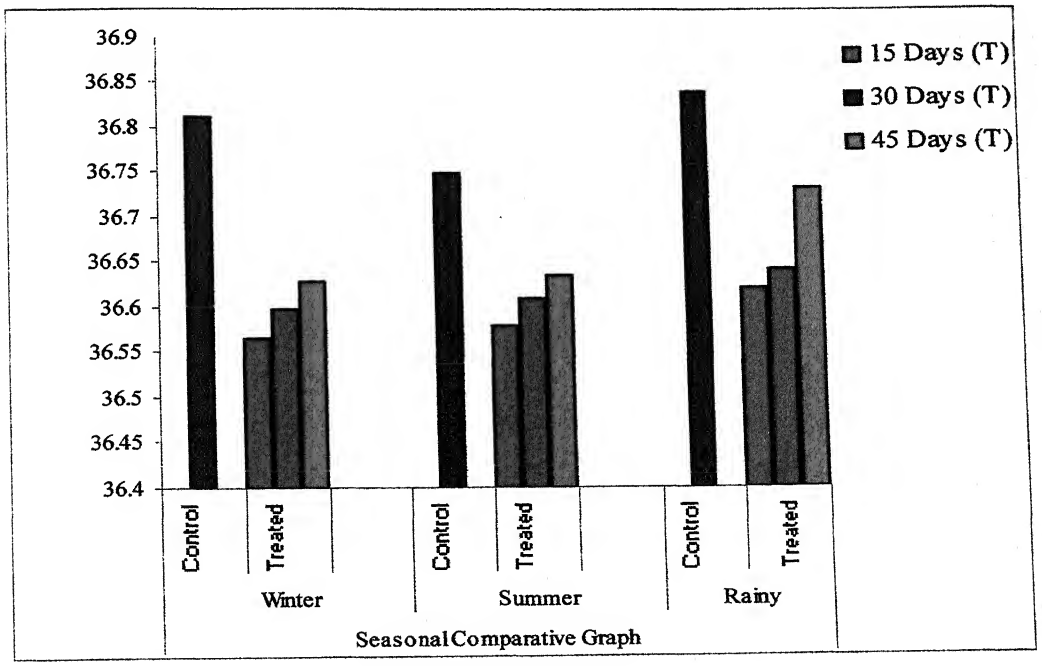


Figure 77. MCH

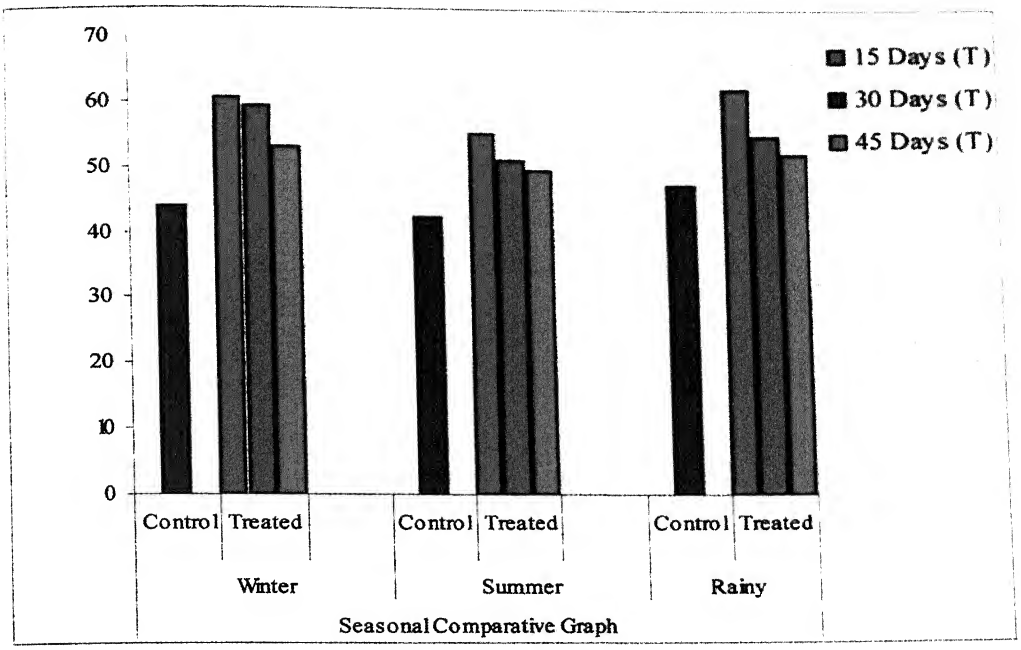


Figure 78. MCHC

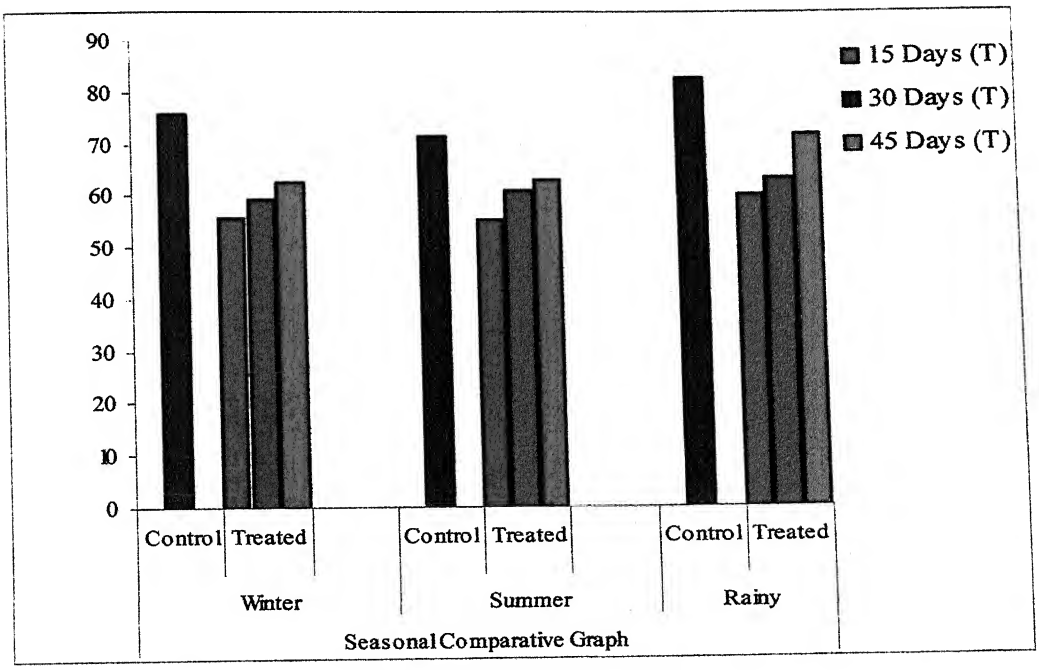


Figure 79. MCV

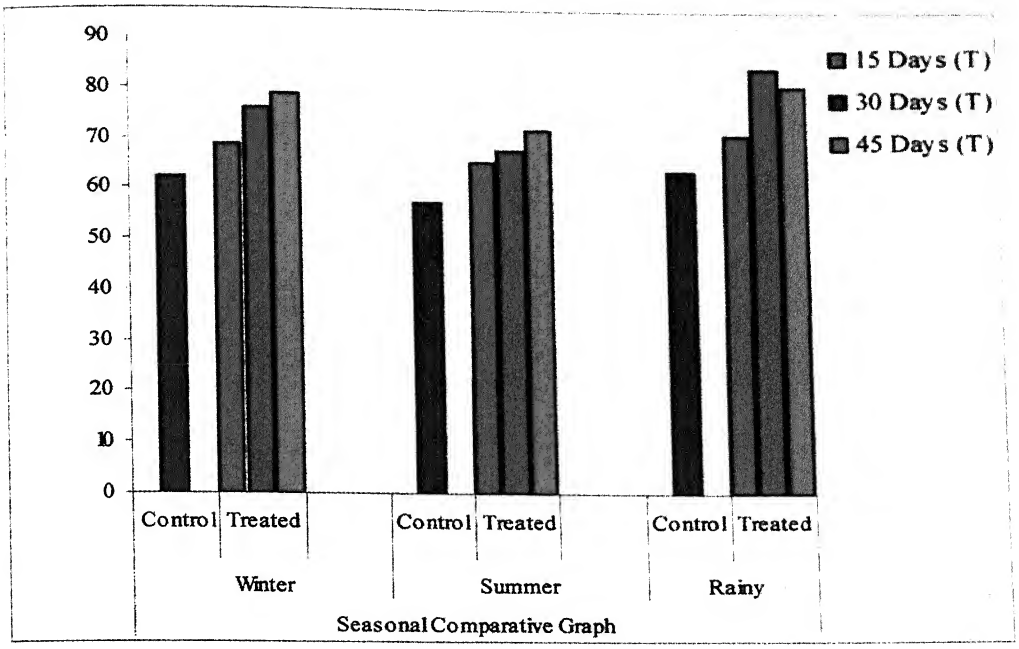


Figure 80. Blood glucose

- **SUMMARY**
- **BIBLIOGRAPHY**
- **ABBREVIATION AND SYMBOLS**
- **ANNEXURE**

SUMMARY

Aquatic toxicology is concerned with the adverse effects of various toxicants. The pesticides are mostly used for the control of insects and weeds to improve crop yield in agriculture in the area where this study was carried out. The pesticides are finally find their way into the aquatic environment through water run off. These pesticides have been reported to negative consequence on biota and the environment at large scale. The pesticides have deleterious effects not only on target organisms but also on non target organisms like fishes. The healthy fishes are much affected by these pesticides because they are highly sensitive to the pesticides. Bundelkhand has rich sources of water reservoir as Betwa river, Cane river, Matatila dam and Pahunj river. These reservoirs have become rich sources of fish farming and valuable source of fish food reaching to different areas of our country. The *Clarias batrachus, Labeo rohita, Heteropneustes fossilis and Channa punctatus* species are some fishes mainly used for food. These fishes are continuously challenged by toxicants used in this area. Keeping this in view four toxicants one Glyphosate (herbicide) and three oraganophosphorus pesticides viz Phosphamidon, Metasystox and Imidacloprid were selected to evaluate data on their toxicity in fresh water fishes *Channa punctatus* of Bundelkhand region. Studies have been shown that when the water quality is affected by toxicants, any physiological changes will be reflected by the haematological and biochemical parameters in fishes. So it is necessary to investigate that how much concentration is required to cause adverse effects of fishes, what blood components are affected by the chemical pesticides and what blood functions are

impaired. Therefore, the aim of this work is the determination of LC_{50} of different toxicants (Glyphosate, Phosphamidon, Metasystox and Imidacloprid) and their effects on blood parameters of *Channa punctatus*. The teleost fish *Channa punctatus* was selected in this study because of its wide availability and edibility in India. The literature survey also confers that very little work has been conducted on these toxicants. The toxicological studies in fresh water fishes *Channa punctatus* following exposure of glyphosate and imidacloprid are conducted for the first time in the present thesis.

Chlorine free tap water was used through out the course of the experiment. The physiological characters of water sample like the temperature of the test medium, dissolve oxygen, alkinity, hardness and specific conductivity were tested in the Zonal laboratory U.P. Jal Nigam Babina, Jhansi. The physiological characters of water during all three seasons rainy, summer and winter respectively were pH 7.4, 6.8 and 7.2,dissolve oxygen 7.6, 6.2 and 8.9 mg/liter, alkinity 326, 320 and 308 mg/liter as $CaCO_3$, hardness 120, 11.7 and 128.7 mg/liter as Ca, specific conductivity 792, 765 and 782 micro mho.

The fishes (*Channa punctatus*) were collected from different water bodies of Bundelkhand region with the help of professional fisher man. Live and healthy fishes were used in all the toxicological investigations. The selected fishes were checked against injury or infection by keeping 0.2% of potassium permanganate solution for 1-2 minute. They were kept in glass aquaria having a capacity of more than 40 liters. All over the experiment water was changed daily. The fishes were acclimatized in laboratory condition for 6-10 days. During acclimatization the fishes were fed egg albumin, earth worms and

small insects.

Under acute toxicity study LC $_{50}$ values after 24h, 48h, 72h and 96h were determined by direct interpolation method, which includes two exploratory and a definitive test. The mortality was recorded after a period of 24, 48, 72 and 96 hrs and dead fishes were removed when observed. In first exploratory test (two concentrations lower and higher) were employed in jar containing five fishes each. Then four and five concentrations were taken to find out narrow range of concentrations in second exploratory test. On the basis of second range finding test 7-9 concentrations were selected for definitive test. The concentrations from the definitive test were employed to determine the LC_{50} values by plotting a dose response curve between percent mortality and concentrations of toxicants. A line was drown between the point represent the % mortality and concentrations. The concentrations at which this line crosses for the 50% lethality line was the actual lethal concentration of toxicant. Therefore the LC_{50} values of all the selected toxicants (Glyphosate, Phosphamidon, Metasystox and Imidacloprid) were determined:-

- The LC_{50} values of Glyphosate in *Channa punctatus* conducted to be as 0.018, 0.015, 0.012 and 0.009 ml/liter at 24, 48, 72 and 96 hours.
- The LC_{50} values of Phosphamidon in *Channa punctatus* were 0.023, 0.019, 0.015 and 0.011 ml/liter at 24, 48, 72 and 96 hours.
- The LC_{50} values of Metasystox in *Channa punctatus* were found to be as 0.034, 0.030, 0.026 and 0.022 ml/liter at 24, 48,

72 and 96 hours.

- The LC_{50} values of Imidacloprid were 0.058, 0.050, 0.042 and 0.034 ml/liter at 24, 48, 72 and 96 hours in *Channa punctatus.*

After calculating the LC_{50} values 40 fishes were collected, acclimatized and divided into 5 groups (A, B, C, D and E) for acute toxicity bioassay. LC_{50} concentrations of selected toxicants were added to the groups A, B, C and D respectively. The fifth group was running without toxicant served as control. The blood was collected after 24, 48, 72 and 96 hours respectively from each group, serum was separated and haematological and biochemical parameters were tested.

For chronic toxicity test 60 fishes were collected from market and washed with 0.2% $KMnO_4$ to avoid any dermal infection and acclimatized for at least 10 days in laboratory condition. The fishes were divided into two groups of 30 each. Group one was exposed to sub lethal concentration (1/10 of 96 hours LC_{50}) of toxicants. The second group was kept as untreated control. After 15, 30 and 45 days 10 fishes from each group were sacrificed, blood was collected and serum was separated for the testing of blood parameters. The whole technique of acute and chronic toxicity bioassay were applied for all the toxicants. All the acute and chronic (blood parameters examinations) studies were also conducted in different seasons viz winter, summer and rainy season.

Glyphosate:-

In acute toxicity test Hb % and TEC were decreased significantly ($P < 0.001$). A significant decrease was also observed in

levels of PCV, MCH & MCV at ($P < 0.01$). The other parameters like TLC and MCHC levels increased significantly at ($P < 0.01$). ESR was also increased from the control fishes. The level of glucose was increased significantly ($P < 0.01$) in treated fishes.

In chronic toxicity bioassay the Hb %, TEC, PCV, MCH & MCV decreased significantly ($P < 0.01$) after 15 days, 30 days and 45 days exposure of Glyphosate. Significant increased in the level of TLC and MCHC was observed ($P < 0.01$). Although an increased level of ESR was observed when compared with the unexposed fishes but statistically the difference was not significant. Glucose level were elevated significantly ($P < 0.01$) when the fishes were subjected to glyphosate treatment.

Phosphamidon:-

The Hb % TEC were decreased significantly $P < 0.001$. Although the values of Hb% and TEC were all the times lesser than untreated fishes but at 72 hours the total erythrocytes counts was insignificant. The values of ESR were insignificantly increased, but at 96 hours it was significant ($P < 0.01$). The data illustrated that the value of PCV, MCH and MCV were significantly decreased ($P < 0.01$). TLC and MCHC were found to increase at $P < 0.01$. The glucose level estimated intreated fishes were markedly increased after all exposure periods.

In chronic toxicity bioassay the levels of Hb % TEC, PCV, MCH & MCV were decreased after 15 days, 30 days and 45 days exposure at 1/10 of 96 hours of phosphamidon LC_{50} concentration. It was observed that the levels of Hb%, TEC, MCH, PCV and MCV

were less than control fishes, but gradually increased with rising exposure periods. The blood parametric levels of TLC, ESR and MCHC were increased significantly. The level of blood glucose was increased significantly at $P < 0.01$. It was lower during 15 days but higher in 45 days exposure period.

Metasystox:-

In acute toxicity test the Hb% and TEC were increased significantly after 24, 48, 72 and 96 hours exposure periods at $P < 0.01$ and $P < 0.001$. The values of PCV, MCH and MCV were significantly increased at $P < 0.01$ and $P < 0.001$. TLC, ESR and MCHC were decreased significantly. The biochemical parameters like glucose level also increased in comparison to control fishes. The levels of glucose increased significantly at $P < 0.01$ in treated fishes.

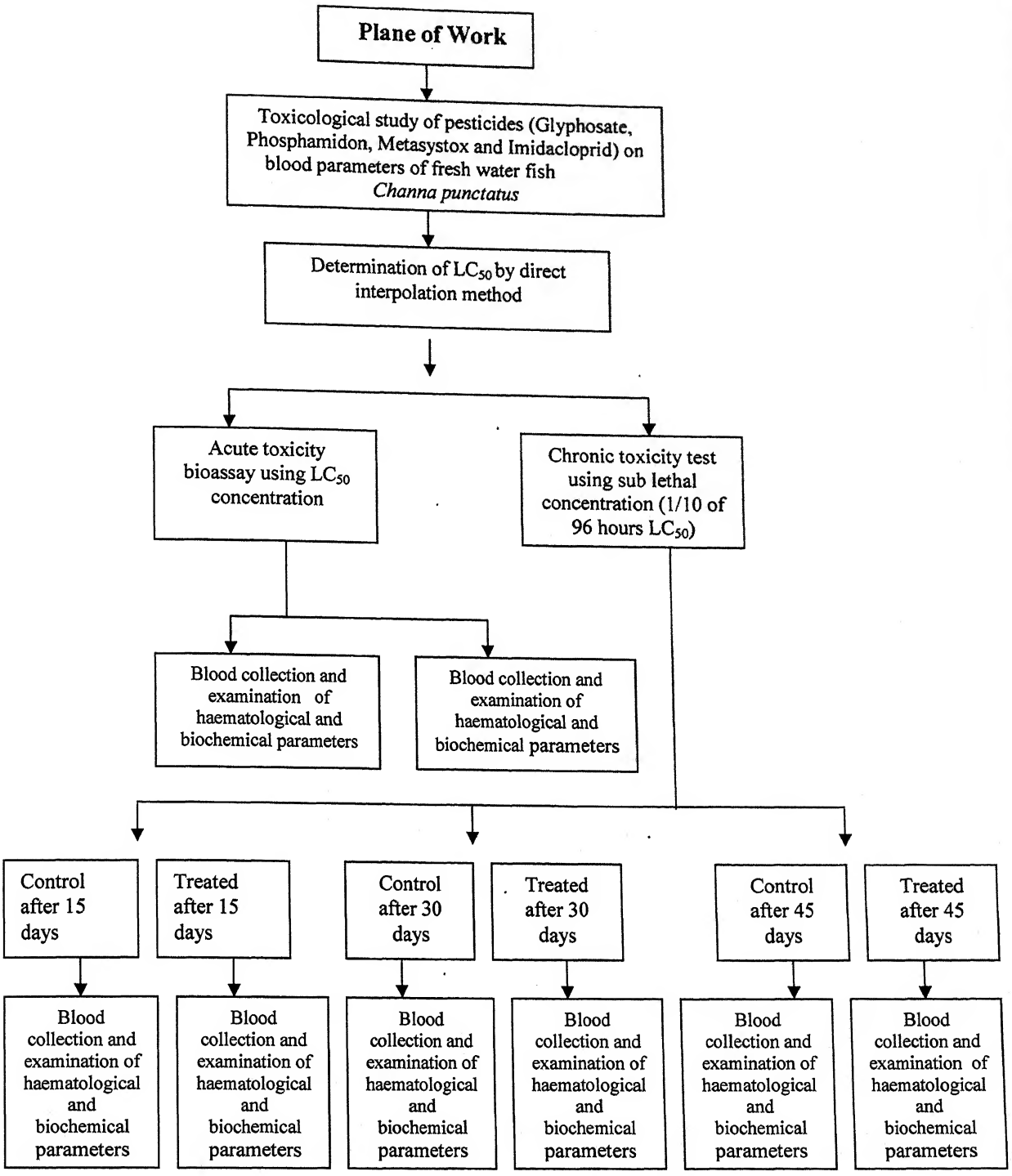
During chronic test the levels of Hb%, TEC, PCV, MCH and MCV were significantly increased at $P < 0.01$ in treated fishes than untreated ones after the end of 15 days, 30 days and 45 days intoxication of metasystox. A significant decreased level of TLC, ESR and MCHC were observed at $P < 0.01$ and $P < 0.001$. The blood glucose level was increased significantly in the insecticidal treated fishes.

Imidacloprid:-

In case of imidacloprid same results were observed as glyphosate and phosphamidon i.e. Hb% TLC, PCV, MCH and MCV decreased significantly while TLC, ESR, MCHC and glucose were increased after 24, 48, 72 and 96 hours exposures.

In chronic toxicity bioassay the Hb % TEC, PCV & MCH decreased significantly after 15 days, 30 days and 45 days exposure of imidacloprid (1/10 of 96 hours LC_{50} concentration). The level of TEC was decreased but no significant difference was observed after the end of 15 and 30 days of exposure periods. A statistically insignificant increased was observed in erythrocytes sedimentation rate expect 15 days exposure period which was significant at $P < 0.001$. The level of blood glucose was increased significantly ($P < 0.01$) in treated fishes.

Fishes lives in very intimate contact with their environment. They are susceptible to physical and chemical changes which may be reflected in their blood components. The changes in water quality can alter the haematological and biochemical parameters. Water temperature, O_2, pH, and other factors also causes disturbance in metabolic rate. Hence this study was also performed to obtain data on seasonal changes in blood parameters of fresh water fishes *Channa punctatus*. If only control fishes were taken into consideration, it was found that Hb%, TEC, TLC, PCV, ESR, MCV, MCH, MCHC and blood glucose level were minimum during summer and maximum during rainy season. When compared to untreated control using Glyphosate, Phosphamidon, Metasystox and Imidacloprid. The activity of Hb%, TEC, PCV, MCH and MCV were decreased while TLC, ESR, MCHC and blood glucose were increased. In case of metasystox treated fishes Hb%, TEC, PCV, MCV and MCH were increased but TLC, ESR and MCHC were decreased. Blood glucose level was also significantly increased. It was found that activity levels of all the parameters were decreased during summer season comparatively.



*All the acute and chronic toxicity experiments were carried out during winter, summer and rainy seasons. Comparisons of different haematological and bio chemical parameters were presented graphically among different season.

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ABBREVIATIONS AND SYMBOLS

1. < smaller than
2. = equal to
3. µg Micro gram
4. Ach acetylcholine
5. AChE acetylcholine enzyme
6. BOD Biological oxygen demand
7. Cas catecholamine
8. cm centimeter
9. CNS central nervous system
10. DO dissolved oxygen
11. ESR Erythrocytes sedimentation rate
12. FAO Food and Agriculture Organization of the United Nations
13. Hb% Haemoglobin percentage
14. L liter
15. LC_{50} lethal concentration, 50% kill
16. MCH Mean Corpuscular Haemoglobin
17. MCHC Mean corpuscle haemoglobin concentration
18. MCV Mean Corpuscular Volume
19. ml milliliter
20. MW molecular weight
21. nAChR Nicotinic acetylcholine receptor
22. ODM Oxydemeton-methyl
23. PCV Packed cells volume
24. PEP phosphoenolpyruvate
25. ppm parts per million

26. r.p.m. Revolution per minute
27. RBC red blood cells
28. TEC Total erythrocytes count
29. TLC Total leucocytes count
30. Unit (blood) (mg/dl)
31. WHO World Health Organization
32. viz videlicet (namely)
33. i.e. id est (that is)
34. a.e. acid equivalents
35. a.i. active ingredient

ANNEXURE

Publications

K. Zahra and Saurabh Shreshth (2005): Variation in blood parameters due to Glyphosate on fresh water fishes *Heteropneustes fossilies*.16Th All India congress of Zoology and national symposium recent advance in animal research with special emphasis on invertebrates. October pp: 96.

K. Zahra and Saurabh Shreshth (2005): Haematological effects in *Channa punctatus* after acute and sub acute exposure of Glyphosate. 16Th All India congress of Zoology and national seminar. December 07: PS 361.

K. Zahra and Saurabh Shreshth (2006): Effect of glyphosate on various blood parameters of fresh water fishes, *Heteropneustes fossilies*. Flora and fauna Vol. 12 No. 1 pp 100-104.